## 3. Results and Discussion

#### 3.1. Identification and characterization of nanomaterial

### 3.1.1 FT-IR analysis

# **3.1.1.1 FT-IR** spectra of graphene oxide (GO) and reduced graphene oxide (RGO).

The chemical composition of GO and RGO has been characterized with FT-IR measurement. The FT-IR Fig. (3-1) shows the spectrum of GO. The stretching vibration of (C-OH, COOH, and residue of H<sub>2</sub>O) appeared at 3401.4cm<sup>-1</sup> with broad and strong band. The peak at 1587cm<sup>-1</sup> was assigned to unoxidized graphitic domain .The two bands at 1221.78 cm<sup>-1</sup> and 1400.5 cm<sup>-1</sup> were corresponded to (C-O) stretching vibration of epoxide groups (C-O-C) and (C-O-H) bending vibration of COOH group or C-OH group respectively.

The OH bending vibration of COOH groups can be used to evaluate the amount of COOH groups [169]. Vibration at 1060.18 cm<sup>-1</sup> was assigned to alkoxy (C-OH) groups. Besides, the band at 1721 cm<sup>-1</sup> might referred to not only the carbonyl group stretching vibration of COOH situated at the edges but also to ketones or quinone [170].

Reduced graphene oxide (RGO) Fig. (3-2) showed that, the O-H stretching vibration peak at 3445cm<sup>-1</sup> was significantly reduced in intensity indicated that C-OH was still existed but in lower proportion. The peak at 1726 cm<sup>-1</sup> was assigned to carbonyl groups of carboxylic groups which hydrazine didn't remove it [171]. The bands remained at 1215 cm<sup>-1</sup> and 1084 cm<sup>-1</sup> showed that even after reduction process, some residual oxygen groups were present may be due to the carboxylic groups. A peak at 1574cm<sup>-1</sup> was assigned to conjugate double bonds(c=c).





Figure (3-1) spectrum of GO



Figure (3-2) FT-IR spectrum of RGO



#### **3.1.1.2 FT-IR of thiocarbohydrazide (TCH)**

Fig. (3-3) shows FT-IR spectrum of prepared TCH .The peaks at 3273.2, 3209.5, 3305.9 cm<sup>-1</sup>corssponded to N-H and NH<sub>2</sub> stretching vibrations. The NH<sub>2</sub> bending and wagging vibrations contributed to the two peaks at 1643.35 and 1143.79 cm<sup>-1</sup> respectively [172]. The characteristic peaks 1531 and1500.6 cm<sup>-1</sup> are assigned to the coupled modes N-H wagging and C-N stretching vibrations [172].The C=S stretching contributes to two peaks at 1288.45 and 935.48 cm<sup>-1</sup> also these peaks contain contributions of other vibration such as C-N stretching and C-N stretching at C-N stretc



Figure (3-3) FT-IR spectrum of TCH

#### 3.1.1.3 FT-IR of functionalize GO and RGO with TCH

The infrared spectrum of GO functionalized with thiocarbohydrazide (GOT) was shown in Fig. (3-4) the peaks in the region 3000-3500 referred to stretching vibration of NH<sub>2</sub> groups, these peaks were not shown in the FT-IR spectra of GO and it was suggested the formation of functionalization of GO with TCH. A band in the 1590-1650 cm<sup>-1</sup> region is characteristic of the NH<sub>2</sub> scissoring vibration; additionally, absorption band at 750-850cm<sup>-1</sup> assigned to NH<sub>2</sub> twisting and wagging deformations. Also , absorption bands at 1333.32, 1501.4, 1175cm<sup>-1</sup> that appeared in spectrum of (GOT)are correspond to stretching vibration of (C=S) ,



C=N, and N-N respectively which denoted to stretching vibration that had been introduced to GO by functionalized with TCH.

The FT-IR spectrum of RGO functionalized with TCH (RGOT) Fig. (3-5) exhibit a band at 3286.7 cm<sup>-1</sup> and 3172.9 cm<sup>-1</sup> attributed to the NH<sub>2</sub> asymmetric and symmetric stretching vibration. The presence of C=S absorption band at about 1296 cm<sup>-1</sup> for thion and characteristic SH absorption band at about 2600-2550 cm<sup>-1</sup> for thiol forms confirm that the thion-thiol tautomeric form was exist. The band at 1583.5 cm<sup>-1</sup> was assignable to stretching vibration of azomethane linkage. The new peaks appeared in the spectra of RGOT confirm that cyclocondensation of TCH with carboxylic groups attached at the edge of RGO sheet was happened.



Figure (3-4) FT-IR spectrum of GO functionalized with TCH.



Figure (3-5) FT-IR spectrum of RGO functionalized with TCH



The hydrogen atom may be bonded either to the sulfur atoms or to nitrogen atom so triazole functionalized with GO or RGO can exist in two forms thion (a) and thiol (b) tautomeric as shown in Fig.(3-6) where x is (RGO or GO)



Figure (3-6) Thion-thiol tautomeric forms

# 3.1.1.4 FT-IR of MnO<sub>2</sub> nanoparticles

Generally, oxide and hydroxide of metal nanoparticles give absorption peaks in the finger print region below wavelength of 1000 cm<sup>-1</sup>arising from inter-atomic vibration. The peaks appeared in Fig. (3-7) at 526 cm<sup>-1</sup> and 480 cm<sup>-1</sup> were assigned to the Mn-O bond [173]. Beside, a peak at 716 cm<sup>-1</sup> referred to stretching mode of MnO<sub>6</sub> octahedral along the double chain [174,175]. These peaks suggested that the  $\alpha$ -MnO<sub>2</sub> was obtained. The absorption peak observed in 3383 cm<sup>-1</sup> can be attributed to expose to the ambient water vapor. The bands at 1635 cm<sup>-1</sup> and 1074 cm<sup>-1</sup> are attributed to the O-H bending vibration.



Figure (3-7) FT-IR spectrum of MnO<sub>2</sub>



# 3.1.1.5 FT-IR spectra of nanocomposites GOT and RGOT with MnO<sub>2</sub>nanopatricles

Fig. (3-8) and (3-9) showed FT-IR spectra for functionalized GOT and RGOT with  $MnO_2$  respectively. Both spectra showed all stretching vibration of GOT and RGOT in lower intensity and appearance of the stretching of Mn-O bonds in  $MnO_6$  octahedra in the region (450–750 cm<sup>-1</sup>) [176, 177].



Figure (3-8) FT-IR spectrum of GOTM nanocomposite



Figure (3-9) FT-IR spectrum of RGOTM nanocomposite.



#### 3.1.1.6 FT-IR of MWCNT and f-MWCNT

Acomparison between MWCNT without treatment Fig. (3-10) and acidic functionalized of MWCNT (f-MWCNT) Fig. (3-11) were shown significant differences between the two spectra, presence the strong peak at1739cm<sup>-1</sup>[178] that corresponded to stretching vibration of carboxylic group indicated clearly that carboxylic group present in the surface of MWCNT is due to the success of the carbon atoms oxidation [179]. The peak at 3741 cm<sup>-1</sup> was assigned to free OH groups, and the peak at 3448 cm<sup>-1</sup>can be attributed to the O-H vibration of carboxyl groups (O=C–OH) and C–OH. Appearance of peak at 1360 cm<sup>-1</sup> may be related with O–H bending deformation in COOH groups' also increased strength of the signal at 1219 cm<sup>-1</sup> may be related with C–O stretching vibration in the carboxylic groups [180]. The peaks at 1547 cm<sup>-1</sup> in the figure (3-10) and 1512 cm<sup>-1</sup> in the figure (3-11) associated with the stretching vibration of the CNT backbone [180]. Which shifted to lower wave number after chemical treatment with the acid mixture.



Figure (3-10) FT-IR spectrum of MWCNT without treatment

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Figure (3-11) FT-IR spectrum of acidic f-MWCNT.

#### 3.1.1.7 FT-IR of f-MWCNT-GOT nanocomposite

Fig. (3-12) of f-MWCNT-GOT nanocomposite showed a broad peak at 3440 which confirms formation of hydrogen bonding between carboxylic groups of f-MWCNT and functional groups of GOT. The absorption of carbonyl group, OH bending, C–O stretching vibration in the carboxylic groups and aromatic C=C bonds are still presence but at slightly lower wave number, due to formation the nanocomposites f-MWCNT-GOT.





Figure (3-12) FT-IR spectrum of f-MWCNT-GOT nanocomposite

#### **3.2 Elemental analysis**

The elemental analysis was carried out in type Euro EA3000 for RGOT and GOT (using 1 mg of sample) as shown in table (3-1). The results indicated the high purity of prepared carbon nanomaterials, and showed the hydrogen percentage in the RGO less than in the GO this referred to the formation of the double bond between the C-C atoms and the removing of OH groups in the middle of the sheets, also the nitrogen was present in a very small percentage (a residue from the preparation process). The presence of sulfur and the increase of percentage of nitrogen in RGOT and GOT indicated that the functionalization happened as shown in table (3-1).

sample	C%	H%	N%	<b>S%</b>
RGO	$75.3 \pm 0.41$	$1.38\pm0.05$	$3.70 \pm 0.04$	-
RGOT	69.30	1.005	17.53	5.288
GO	$55.09 \pm 0.14$	$2.55 \pm 0.05$	$0.12 \pm 0.006$	-
Ref [22] GO	42	2.809	-	1.575
GOT	55.914	1.783	22.04	8.260

Table (3-1) elemental analysis of GO, RGO, GOT and RGOT



# 3.3 XRD analysis

## 3.3.1 XRD of graphite GT, GO and RGO

XRD patterns of graphite flake GT Fig. (3-13) show distinct diffraction peak at  $26.4^{\circ}$  (d= 0.336 nm) [181], 43.36° and 54.54° correspond to planes (002), (101) and (004) respectively. The XDR for GT was compared with reference card [No.00-056-0159c] and with literature data (JCPDS 75-2078, 3.347 Å) [181] and we found the position and intensity of the reference matched with data with very small amount of mismatch due to experimental errors.



Figure (3-13) XRD pattern of GT

A powder sample of GO was examined with x-rays diffractometer Fig. (3-14) shows the XRD pattern of GO. It is found that GO exhibits a strong diffraction at 11.95° corresponding to d-spacing of 0.73 nm [182] [181]which is higher than that of graphite (0.336 nm) indicated introduce functional groups (carboxylic acid, carbonyl, hydroxyl and epoxy ) on the basal and edges planes of GO sheets [183] Moreover, the increase in d-spacing proved the oxidation of graphite flakes (GT) Since the degree of oxidation is proportional to the interlayer spacing (d) of GO [184, 185].

The thickness of GO sheets (t) was calculated using the classical Debye–Scherrer.

$$t = \lambda \, k / \beta \cos\theta \tag{3-1}$$



Where k is the Scherrer constant depends on the shape of the crystal, and the size distribution (shape factor).  $\beta$  is the angular full width at half maximum peak(FWHM) intensity in radian, ( $\lambda = 0.1541$  nm) for Cu K $\alpha$  radiation source and  $\theta$  is the Bragg angle (deg) .the diffractograms were recorded in range of 10-80°.The thickness was found to be 6.6 nm. The numbers of layers stacked (n) can be estimated from the following relation

n = t/d

(3-2)

Where d is the interlayer spacing .the number of perpendicular stacked GO sheets was found to be 9 as shown in height profile obtained from AFM measurement Fig (3-21). The value of thickness and the number of layers were lower than values obtained in the literature [186].The peaks at  $26.5^{\circ}$  and  $44.3^{\circ}$  and it's d-spacing, resemble the one of pristine graphite which indicated that trace of starting material was present in the sample [181]



#### Figure (3-14) XRD patterns of GO

The XRD patterns of RGO Fig. (3-15) show a broad diffraction peak appeared at  $2\theta 23.7^{\circ}$  [187] indicates poor ordering of the sheets along the stacking direction, which implies the sample was comprised mainly from a few layers of RGO and 43.6  $^{\circ}$  corresponding to(002) and (100) planes of RGO respectively, with an



interlayer d-spacing of 0.4805 nm which is smaller than GO due to the removal of most oxygen functional groups [188] as indicated in the FT-IR results. The interlayer d-spacing of RGO was little higher than graphite GT may be attributed to remaining some of oxygen functional group, referring to partial reduction of GTO nanoplates by hydrazine, the appearance of this peak and the disappearance of the diffraction of GO (2 theta 11.95°) implying that the RGO nanosheets were formed, and result a new lattice structure. The thickness or height and the numbers of layers stacked (n) were also estimated and found to be 3.699 nm and 7 respectively. This number of stacked layers referred to graphene nanoplatlets which have distinct properties [189] Reduced graphene oxide (graphene nanoplatlets) is considered as intermediate phase between graphite and pristin graphene known as *quasi*-graphene [190]





# 3.3.2 XRD OF thiocarbohydrazide (TCH).

Fig. (3-16) shows the XRD of TCH. Comparing the diffraction peak of TCH with known standards in the JCPDS file [35-1800] showed the presence of diffraction peaks in the same position with very small variation in intensity.





Figure (3-16) XRD of TCH

# 3.3.3 XRD of functionalize GO and RGO with TCH

The XRD pattern of the prepared GOT showed the appearing of many other miller indices due to the TCH crystallinity structure Fig.(3-17) and also a decrease in the intensity of GO at  $20 44.3^{\circ}$ . On other hand a decrease in the d-spacing of GO from 0.739 nm to 0.688 nm confirmed functionalize of GO with TCH and indicated the formation of a new lattice structure. Also, there was an emergence of the new well-defined Bragg reflections at 20 angles in the diffraction patterns which suggests that the GOT was formed.



Figure (3-17) XRD of GOT



The XRD pattern for RGOT have a different diffraction intensity from RGO as shown in Fig. (3-18) and the chemicals functionalization of TCH molecules onto RGO gives rise to decrease the d-spacing from 0.364 nm to 0.354 nm.



Figure (3-18) XRD of RGOT

#### 3.3.4 XRD of MnO<sub>2</sub> nanoparticles

The XRD pattern of MnO<sub>2</sub> is shown in the Fig. (3-19). the diffraction that appeared at  $2\theta = 12.69$ , 18.1, 28.75, 37.5, 42.02, 49.8, 56.4 and 60.09 were matched well with the diffraction peak of crystal planes of  $\alpha$  –MnO<sub>2</sub> standard data (JCPDS card No.44-0141) [131]. No peaks of other phases are detected, indicating the high purity of the synthesized product. Crystalline size for the powder sample obtained at 25 <sup>o</sup>C was estimated according to the Scherrer equation using the diffraction of maximum intensity.

The diameter of the MnO<sub>2</sub> nanorod was calculated to be (11.8 nm) using the strongest diffraction peak of (211) and by substituting the diffraction angle  $\theta$  (18.7766°) and  $\beta$  (0.0124 rad) in the Debye –scherrer equation. This value was corresponding with the value observed by AFM. Increasing number of mole of KMnO<sub>4</sub> in the reaction mixture plays important role in decreasing average diameter of nanorods. According to the literature [191] using 6mmole of KMnO<sub>4</sub> made the diameter of nanorods 20nm.





Figure (3-19) XDR of MnO<sub>2</sub> nanoparticles

# 3.3.5 XRD OF GOTM and RGOTM nanocomposites

The XRD pattern Fig. (3-20) represented the diffractograms of RGOTM .its diffraction showed a good similarity to the GOTM Fig. (3-21), except its peaks were with more intensity. The peaks at  $2\theta$ = 37 (211), 66 (002) and 54.7(600) indexed to  $\alpha$ -MnO2 (JCPDS No. 44-0141)



Figure (3-20) XRD of RGOTM nanocomposites





Figure (3-21) XRD of GOTM nanocomposites

#### 3.3.6 XRD of functionalized f-MWCNT

X-ray diffractions could provide useful information on the structural properties of MWCNT based material [192].Fig (3-22) showed the XRD pattern of f- MWCNT. XRD showed strong diffraction peaks at  $2\theta = 26.2^{\circ}$  in addition to two diffractions at 43° and 54° coresponding to the (002), (100) and (004) planes respectively of graphitic structure of MWCNT [193 - 194]. The peaks of the functionalized MWCNT are very sharped and intense, indicating the graphitic structure of MWCNT have excellent crystallinity (without damaged). The XRD of CNT and graphite GT is very similar due to XRD method can disclose the graphitic structure of carbon and these results were consistent with the reference [195].



Figure (3-22) XRD of f-MWCNT

#### 3.3.7 XRD of f-MWCNT-GOT composite

Fig. (3-23) showed the XRD pattern of f-MWCNT-GOT nanocomposite, the peak at  $2\theta = 26.4^{\circ}$  was relevant to the characteristic of MWCNT. It was also observed that GOT didn't shift the plane (002) of graphite, the distance between planes is little increased (0.337 nm).Presence of GOT nanosheets affected the peak intensity of the f-MWCNT and caused the growth of new diffraction peaks (low intensity) indicated the f-MWCNT-GOT nanocomposites were synthesized.



Figure (3-23) XRD of f-MWCNT-GOT nanocomposites

#### 3.4 AFM measurement

#### 3.4.1 AFM of GO

AFM was a good technique to give real images for the surface morphology, two dimensional (2D) image for GO sheets observed many sheets, the area A in the cross section at the black arrow in the Fig. 3-24 (A) represented a double layers sheets with thickness about 5.94 nm Fig. (3-24B), also Fig. (3-24B) show the B area which represented the area of mono layer sheet with thickness 3.71nm. Three dimensional (3D) image was shown in Fig. (3-24C) and demonstrated the morphology of the surface and showed the difference in the high between the single and double layers. On other hand these images indicated good chemical



preparation of GO material, which consisted one and two layers sheet. The average lateral size of overall GO flakes is  $1.25 \mu m$ 



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Figure (3-24) AFM imaged for GO sheets (A) image with two mono and double layers 2D (B) A cross section of the image (C) Three dimensional image 3D.

# 3.4.2 AFM for RGO

AFM was also used to examine the morphology and the thickness of the RGO sheets. As shown in Fig. (3-25A, B and C). Figure A showed the 2D image and appeared the RGO sheets with total accumulation reached to 33.4nm, the cross-section at the black arrow in the figure A showed the lowest thickness of the accumulated sheets about 12.68 nm figure B, which indicated about 3 sheets one on the others[196]. The three dimension image figure C show almost sheets appear equal in value rate of them clumping above the other.

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Figure (3-25) (A) An AFM image of RGO sheets, (B) thickness profile (cross-section) of RGO sheets marked by the black arrow in (A) and (C) 3D view.

# 3.4.3 AFM MnO<sub>2</sub> nanoparticles

MnO2 nanotubes showed good images in two and three dimensions Fig (3-26A) image showed the growth of nanorods from the body of the spherical  $MnO_2$  nanoparticles and this case it came in accordance with the literature [197].The nanorods appeared very clear with the length ca. 127.1 nm. A cross-section in Fig. (3-26B) of this nanorod was with a diameter of ca. 5.48-12.48 nm appeared, the cross-section was made at the black arrow in the two dimensions(A). This value was a good agreement with value obtained from XRD. Many MnO2 nanorods were shown in the image 3-26 (C).

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Figure (3-26) (A) 2dimensions, (B) cross-section at the black arrow in the two dimensions (A) and (C) Three dimension image of the same MnO2 nanotube.

# 3.5 SEM analysis

Fig. (3-27) Shows SEM images of  $\alpha$ - MnO<sub>2</sub> nanoparticles in micro (A) and nanoscale (B). It is clearly shown that the product is made up of distinctive  $\alpha$ -MnO<sub>2</sub> nanorod (almost equal in length) was observed to initiated from the surface of MnO<sub>2</sub> formed from spherical MnO<sub>2</sub> nanoparticles.

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Figure (3-27) SEM image of MnO<sub>2</sub> nanorods (A) microscale (B) nanoscale

## **3.6 Electrical properties**

The permittivity can change widely between solids. Furthermore, the response of permittivity with frequency of applied field and temperature can be either linear or nonlinear [198]. Electric properties such as real permittivity ( $\epsilon$ ), imaginary permittivity ( $\tilde{\epsilon}$ ) and Alternating electrical conductivity ( $\sigma_{a.c}$ ) were measured for all prepared nanomaterials.

#### **3.6.1 Real permittivity** $(\epsilon')$

The relative permittivity was usually known as dielectric constant, this term was no longer used in chemistry and engineering [199] as well as in physics [200]. Real permittivity ( $\epsilon$ ') characterizes materials to store charge (energy) when the material exposed to an electric field. The capacitance (c) in F was determined by the LCR meter measurements so the permittivity can be calculated using equation below [201]:

$$\varepsilon' = \frac{cd}{\varepsilon_{A}} \tag{3-3}$$

Where ( $\varepsilon$ ), (d), and (A) were the permittivity of free space (8.854x10<sup>-12</sup>) F.m<sup>-1</sup>, thickness of the sample (m) and the area of the electrode (m<sup>2</sup>) respectively Table (3-2) and (3-3), show the values of real permittivity of GO, RGO and their nanocomposites.



Frequency(Hz)	Log f	<b>Real Permittivity</b> (ε')		
		GO	GOT	GOTM
100	2	32090.4	51086252	28166855
120	2.1	26636.53	46467043	23696422
200	2.3	14463.28	25031714	13436309
400	2.6	8376.64	27187345	5970998
1000	3	3397.36	3013484	3266855
2000	3.3	1729.56	1.64	1254847
4000	3.6	900.94	1.14	1250158
10000	4	386.8927	2.96	321371
20000	4.3	213.93	0.62	114543.1
40000	4.6	125.49	708.27	126704
100000	5	69.54	717.62	61085.5

Table (3-2) The real permittivity of GO and its nanocomposites

Table (3-3) The real permittivity of RGO their nanocomposites

Frequency(Hz)	Log	<b>Real Permittivity</b> (ε')		
	f	RGO	RGOT	RGOTM
100	2	45657250.47	33111864.41	0.47
120	2.1	31658606.4	27593220.34	0.65
200	2.3	20779510.36	16564293.79	0.53
400	2.6	8598418.07	8290508.47	0.45
1000	3	2796233.52	3143954.80	0.39
2000	3.3	1512150.65	1.33	99662.52
4000	3.6	720903.95	0.89	59053.48
10000	4	270448.21	0.78	15049416.2
20000	4.3	45049.94	0.53	13742674.2
40000	4.6	50201.12	29934.46	23915254.24
100000	5	69032.01	1199.05	29448587.57

76

The variation of real permittivity ( $\epsilon$ ') of GO, GOT and GOTM with frequency showed a sharp decrease with increase of the frequency till 2 KHz and nearly continued the permittivity in very low range as presented in Fig. (3-28).



Figure (3-28) Real permittivity ( $\epsilon$ ) of GO, GOT, and GOTM versus frequency

Also Fig. (3-29) showed the relation between real permittivity ( $\epsilon$ ') of RGO, RGOT and RGOTM with frequency. The real permittivity of RGO and RGOT shows extremely high positive permittivity values at low frequency and decrease with the increasing of the frequency per contra the RGOTM showed very low values at the low frequency and at 2 KHz the real permittivity began to increase with frequency increasing. This difference in the behavior between the RGO and RGOT and on the other hand the RGOTM, the presence of manganese nanoparticles which may affect the accumulation of the charges and caused the dispersion of the electric charges and reduced the real permittivity values at the low frequency, but at high frequency, the haste of frequency relay was faster than the dispersion of the electrical charge process and whenever the frequency increasing the amount of charge accumulated increases inside the material leaded to an increase in real permittivity.



# Chapter three



Figure (3-29) Real permittivity ( $\varepsilon$ ) of RGO, RGOT, and RGOTM versus frequency.

# **3.6.2 Imaginary permittivity** (ε")

Simply said the imaginary permittivity was quantity of electric energy that transforms into heat energy. The ratio of the imaginary part of the permittivity to the real part of the permittivity [202] was known as loss tangent (tan  $\delta$ ) or the dissipation factor (D) which is shown in the following equation [201].

$$\tan \delta = \varepsilon'' / \varepsilon' \tag{3-4}$$

Rearrangement of equation (3-4) the imaginary permittivity ( $\epsilon$ ") can calculate from equation:

$$\varepsilon'' = \varepsilon' \tan \delta \tag{3-5}$$

The values of imaginary part of permittivity ( $\varepsilon$ ") for GO, GOT and GOTM tabulated in table (3-4) and plotted with frequency Fig. (3-30), these data showed lower but almost high values in comparison with real permittivity values table 3-3. The GOT nanomaterials showed the highest imaginary permittivity which indicated that this carbon nanomaterial had a high conductivity values at least at the low frequency range table 3-3 and fig.3-32.



# Chapter three

<b>Frequency</b> (Hz)	Log f	Imaginary permittivity (ε")		
		GO	GOT	GOTM
100	2	28467.4	48991716.01	21437793.33
120	2.1	27869.8	46480983.43	17672791.41
200	2.3	16712.3	29191984.57	9867625.22
400	2.6	9418.7	30398170.03	6842763.84
1000	3	3464.9	3537830.207	3569692.44
2000	3.3	605.3	0.527909605	1005132.81
4000	3.6	315.3	0.388893409	1259159.33
10000	4	18.6	0.059389831	341681.6
20000	4.3	25.7	0.123657326	98071.8
40000	4.6	148.3	70.11	132684.38
100000	5	103.8	92.57	119929.16

Table (3-4) The imaginary component of permittivity of GO and their nanocomposite



Figure (3-30) the variation imaginary part of permittivity ( $\epsilon$ ") of GO, GOT, GOTM with frequency.

Table (3-5) showed the imaginary component of permittivity of RGO and their nanocomposites. At low frequency the result showed that imaginary permittivity values of RGO have high positive values when compared with RGO and RGOTM. RGOTM have



very low positive values. Also imaginary permittivity of RGO higher than real permittivity may be due to RGO which has high a.c. conductivity. At higher frequency the real permittivity of RGO and RGOTM is higher than imaginary while real and imaginary values of RGOTM have fluctuated behavior.

**Frequency(Hz) Imaginary permittivity** (ε") Log f RGO RGOT **RGOTM** 61751431.26 32101952.54 100 2 0.37 120 56035733.33 2.1 28175437.29 0.45 200 2.3 23707343.37 18000418.06 0.22 400 2.6 9514149.605 8087391.017 0.05 1000 2782811.601 3047435.39 0.03 3 2000 1449850.052 0.42 81593.70 3.3 4000 657320.226 0.27 227769.28 3.6 10000 4 208461.481 0.04 16038162.84 9791655.367 20000 24295.43453 0.08 4.3 40000 29608.62644 17628.40 15482735.59 4.6 100000 5 148405.02 87.65 18119715.93

Table (3-5) The imaginary component of permittivity of RGO and their nanocomposites.



Figure (3-31) The variation imaginary permittivity  $\hat{\epsilon}$  of RGO, RGOT, and RGOTM with frequency.



#### **3.6.3** Alternating electrical conductivity $(\sigma_{a.c})$

The a.c conductivity  $(S.m^{-1})$  of GO, RGO and their nanocomposites films was calculated with varying frequency (f) in Hz ranging from 100 Hz to 100KHz. using the relation [203].

$$\sigma_{a.c} = \omega \varepsilon_{\circ} \dot{\varepsilon} tan \delta$$

(3 - 6)

Where  $\omega$  was the angular frequency which equals to (2 $\pi$ f). Table (3-6) and Fig. (3-32) presented the correlation between the a.c conductivity versus frequency of the GO their nanocomposites respectively.

The ac conductivity of GOT at low frequency was higher than the GO and GOTM and decreased after 400Hz and stayed with very low values after 2KHz, the GOTM showed an increase in the ac conductivity at frequency values more than 20KHz but the GO shown very low values at all frequencies. Functionalize GO with TCH made a.c conductivity for GOT almost equal or greater than a.c. conductivity for RGO at low frequencies.

Frequency(Hz)	Log f	$(\sigma) (S.m^{-1})$		
		GO	GOT	GOTM
100	2	0.00015	0.27	0.119
120	2.1	0.00018	0.30	0.117
200	2.3	0.000185	0.32	0.10
400	2.6	0.0002	0.67	0.15
1000	3	0.00019	0.19	0.19
2000	3.3	6.72E-05	5.86E-08	0.11
4000	3.6	7.01E-05	8.64E-08	0.27
10000	4	1.03E-05	3.30E-08	0.18
20000	4.3	2.85E-05	1.37E-07	0.10
40000	4.6	0.0003	0.00015	0.29
100000	5	0.0005	0.0005	0.66

Table (3-6) the alternative conductivity ( $\sigma_{a,c}$ ) of GO and their composites

# Chapter three



Figure (3-32) Variation of a.c. conductivity ( $\sigma_{ac}$ ) with frequency of GO, GOT and GOTM.

Table and fig (3-7) and (3-33) respectively showed the a.c conductivity for RGO and its composite. The reduced carbone nanomaterials showed a different conductivity behavior than the unreduced materials, the RGO showed relatively higher conductivity at the low frequencies and these conductivity values decrease with increasing of the frequencies, on the other hand the conductivity of RGOT nanomaterials decreased with increasing of the frequencies and stayed at the minimum value in 2KHz.

The RGOTM didn't show any mentioned differences in its conductivity with change of the frequencies in low range of frequencies while its conductivity began to increase from frequency 2KHz and reached maximum at 100 KHz. Table (3-7) The alternative conductivity ( $\sigma_{a,c}$ ) of RGO and their composites

<b>Frequency</b> (Hz)	Log f	$(\sigma) (S.m^{-1})$		
		RGO	RGOT	RGOTM
100	2	0.34	0.17	2.08E-09
120	2.1	0.37	0.18	3.06E-09
200	2.3	0.26	0.20	2.55E-09
400	2.6	0.21	0.17	1.22E-09
1000	3	0.15	0.16	1.67E-09
2000	3.3	0.16	4.75E-08	0.009
4000	3.6	0.14	6.16E-08	0.05
10000	4	0.11	2.53E-08	8.91
20000	4.3	0.02	9.81E-08	10.88
40000	4.6	0.06	0.03	34.41
100000	5	0.82	0.0004	100.70

82



Figure (3-33) Variation of a.c conductivity ( $\sigma_{ac}$ ) with frequency of RGO, RGOT, and RGOTM.

Also the real and imaginary permittivity of the f-MWCNT-GOT nanocomposites material were studied and electrical conductivity for MWCNT and f-MWCNT-GOT nanocomposite were studied within the frequency range of 100 - 100 KHz as showed in Table (3-8). The real permittivity for f-MWCNT-GOT nanocomposites Fig(3-34) started at high positive values at 100Hz then, decreased sharply crosses zero (negative permittivity) and increased at 1 KHz with slightly increasing with increasing frequency. The values of the imaginary permittivity fig. (3-35) were higher than the real values. This difference is due to the high conductivity of the f-MWCNT, this reason caused the leakage of the electric charge from the material and appeared the imaginary permittivity higher than the real permittivity. This behavior was similar to J.Zhu *et.al* [204]. It is interesting that real permittivity of RGO behavior almost similar with MWCNT in the same frequency range (low frequency).



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# **Results and Discussion**

Table (3-8) Real, imaginary permittivity and a.c. conductivity of f-MWCNT-G	OT
nanocomposites and a.c. conductivity of MWCNT.	

Freq.(Hz	Log f	f-MWCNT-GOT			MWCNT
,		Real Permittivity (ε')	<b>Imaginary</b> <b>permittivity</b> (ε")	<b>(σ)</b> (S.m <sup>-1</sup> )	<b>(σ)</b> (S.m <sup>-1</sup> )
100	2	42250847.5	4286770983	23.82	0.020
120	2.1	24474576.3	2787654237	18.59	0.022
200	2.3	13568361.6	1716397740	19.07	0.034
400	2.6	-17346892.7	848436519.8	18.86	0.056
1000	3	-1339661.02	338666305.1	18.82	0.13
2000	3.3	-79177.40	168917067.6	18.77	0.20
4000	3.6	-961807.91	84379407.91	18.75	0.37
10000	4	94463.27	34176813.56	18.99	0.74
20000	4.3	163163.84	17017988.7	18.91	1.11
40000	4.6	123661.01	8519007.458	18.93	1.2
100000	5	76944.63	3370944.362	18.73	2.53



Figure (3-34) Variation of real permittivity ( $\hat{\epsilon}$ ) with frequency of f-MWCNT-GOT nanocomposites.





Figure (3-35) variation of imaginary permittivity  $\hat{\epsilon}$  of f-MWCNT-GOT nanocomposite with frequency.

Fig. (3-36) shows the ac conductivity (s.m<sup>-1</sup>) of nanocomposites f-MWCNT-GOT and MWCNT as function of frequency. The nanocomposites exhibit higher conductivity at 100Hz, with increasing the frequency the conductivity varied little. The conductivity of the composite was higher than the other prepared nanomaterial and MWCNT without functionalized.



Figure (3-36) Variation of a.c conductivity ( $\sigma_{ac}$ ) with frequency of f-MWCNT-GOT nanocomposites.



# **3.7** Electrochemical characterization of the modified SPCE with GO and GOT using ferri-ferro cyanide.

The CV technique was used to investigate the effect of different carbon nanomaterials and theirs functionalization on the oxidation reduction reactions Fig.(3-37) showed the cyclicvoltammograms (CVs) for bare SPCE and modified SPCE with GO and GO functionalzed with (ASTT) (GOT)obtained in 0.1M KCl solution containing 0.5 mM Potassium ferricyanide  $K_3$ [Fe(CN<sub>6</sub>]<sup>-3</sup> at 0.1V.s<sup>-1</sup> scan rate.

Potassium ferricyanide (red crystalline salt contains the octahedral coordinated ion) was chosen as an electroactive probe to account the advantages of the modified electrode [205] and for its electrochemical response particularly for carbon materials (implies a single electron transfer and produce a quasi-reversible outer sphere kinetic behavior) [127]. An optimum potential range was determined by using a wide range (-1.0V to+1.0V) at slow scan rate ( $0.01V.s^{-1}$ ) before performing the measurements on the electrodes. From this experiment, a lower potential range, from -0.3V to +0.6V was selected. In this range the redox peaks of Potassium ferricyanide was appeared [127]. At bare SPCE and modified GO-SPCE well-defined redox peaks were observed and the peak-to-peak separation ( $\Delta E_p$ ) 0.24V with small redox peak current, which was mainly due to slow electron transfer rate at the surface of electrode/solution interface.

At the modified GOT- SPCE the pair of redox peak current of ferricyanide was increased and the  $\Delta E_p$  value decrease to 0.18V due to functionalize (ASTT) at the edge of GO sheet. According to K.R. Kneten and R.L. McCreery, the electron transfer rate is much faster at the edge plane than at the basal plane[206].

The ratio of the anodic and cathodic peak currents reached unity (Ipa/Ipc = 0.983) indicating that GOT could effectively increase the electron transfer rate of potassium ferricyanide. A large peak separation (>300 mV) means a resistance to electron transfer [207] these findings indicate that the fastest electron transfer electrochemical reaction occurs at GOT -SPCE than bare SPCE and GO-SPCE. Thus a significant electrochemical behavior refinement could be carried out in the modification of SPCE with GOT.

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Figure (3-37) CV curves of bare SPCE, GO and GOT-SPCE in 0.1M KCL solution containing 0.5 mM  $K_3$  [Fe (CN)<sub>6</sub>]<sup>-3</sup>at 0.1V.s<sup>-1</sup> scan rate

The effective area of the electrodes a bare SPCE ,GO-SPCE and GOT-SPCE was calculated in a solution of 0.5mM  $K_3$ [Fe(CN)<sub>6</sub>]<sup>-3</sup> in 0.1M KCl, using Randles Sevick equation [208] at different scan rates ranging from 0.01 to 0.1V.s<sup>-1</sup>

$$I_p = 2.69 \times 10^5 \mathrm{A} n^{3/2} D_R^{1/2} c v^{1/2}$$
(3 - 7)

Where (I<sub>p</sub>) refers to the peak current, (A) is the surface area of electrode cm<sup>-2</sup>, n is the number of electrons ,D<sub>R</sub> is diffusion coefficient, c is the concentration of K<sub>3</sub>Fe(CN)<sub>6</sub> and  $\upsilon$  refers to the scan rateVs<sup>-1</sup>.For Potassium ferricyanide , D<sub>R</sub> = 7.6 × 10<sup>-6</sup> cm<sup>2</sup> s<sup>-1</sup>[209],and n=1.The redox behavior of 0.5mM K<sub>3</sub>[Fe(CN)<sub>6</sub>]<sup>-3</sup> in 0.1M KCl was investigated by cyclic voltammetry. The redox peak currents at bare SPCE, GO-SPCE and GOT-SPCE increased linearly with scan rate ranging from 0.01 to 0.1V.s<sup>-1</sup> Figs. (3-38), (3-39) and (3-40) respectively.

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ρ	87	






Figure (3-39) Cyclic voltammogram for GO-SPCE in potassium ferricyanide





Figure (3-40) Cyclic voltammogram for GOT-SPCE in potassium ferricyanide. The current obtained from voltammograms for electrodes recorded and tabulated in Table (3-9).

Scan rate	bare SPCE		GO-SPCE		GOT-SPCE	
Vs <sup>-1</sup>	current µA		current µA		current µA	
	I <sub>Pa</sub>	I <sub>pc</sub>	I <sub>Pa</sub>	I <sub>Pa</sub>	I <sub>Pa</sub>	I <sub>pc</sub>
0.01	3.062	-2.822	2.742	-2.614	2.969	-2.482
0.02	3.952	-4.057	3.726	-4.712	4.147	-3.66
0.04	4.747	-5.126	5.208	-4.920	5.606	-4.738
0.06	5.482	-5.936	6.059	-6.020	6.341	-4.712
0.08	5.797	-6.472	6.716	-6.654	6.939	-4.920
0.1	5.940	-6.801	7.008	-6.913	7.331	-6.032

Table (3-9) Oxidation reduction peaks current for bare, GO and GOT-SPCE at different scan rates

Plotting the  $I_{Pa}$  and  $I_{pc}$  for bare SPCE, GO-SPCE and GOT-SPCE versus  $v^{1/2}$  produced a straight line. Surface areas were calculated from the slope of these linear graphs table (3-10). The apparent electroactive area values was found for the electrodes bare SPCE, modified electrode GO-SPCE and GOT-SPCE to be 0.04 cm<sup>2</sup>, 0.05 cm<sup>2</sup> and 0.06 cm<sup>2</sup> respectively. This enlargement in the effective surface area proved the modification of bare SPCE with GO and GOT.



	Bare-SPCE	GO-SPCE	GOT-SPCE
Slope for $I_{pa}$ vs $\nu^{1/2}$	15.732	20.308	22.012
R <sup>2</sup>	0.9557	0.9837	0.9778
Slope for $I_{pc}$ vs $\nu^{1/2}$	-15.946	-19.625	-23.547
R <sup>2</sup>	0.9821	0.9687	0.982

Table (3-10) Slope and correlation coefficient for plotting redox peaks current vs square root of scan rate

## **3.7.1 Electrochemical behavior of GOT modified SPCE with the antibiotics AMP, AMOX and TET**

The change in the pH value affected the electrochemical oxidation process through changing the proton concentration. The modified electrode GOT-Nafion/SPCE examined with 0.1 mM ampicillin AMP, Amoxicillin AMOX and tetracycline TET in phosphate buffer solution PBS at different pH using cyclic voltammetry CV technique at scan rate 0.1V.s<sup>-1</sup>as shown in Figs. (3-41) and (3-42) there was no redox peaks for AMP or TET respectively at the modified electrode.



Figure (3-41) Cyclic voltammogram of 0.1mM AMP in 0.1M PBS (different pH) at GOT-Nafion/SPCE. Scan rate  $0.1v.s^{-1}$ 





Figure (3-42) Cyclic voltammogram of 0.1mM TET in 0.1M PBS (different pH) at GOT-Nafion/SPCE. Scan rate 0.1v.s<sup>-1</sup>

AMOX showed significant response Fig (3-43). This varying behavior of the modified SPCE with GOT nanomaterial toward these analytes was unclear. The redox peaks current varied with pH values, the redox peak at pH3 was appeared with higher oxidation and reduction current values.



Figure (3-43) Cyclic voltammogram obtained at GOT-Nafion/ SPCE with 0.5mM AMOX in PBS with pH values of 2, 3, 4, 5, 6, 7, and 8 at scan rate 0.1v.s<sup>-1</sup>



There was no response of blank PBS in GOT-Nafion/ SPCE in all pH range as shown in Fig. (3-44) indicated that the current and potential value of the oxidation and reduction were due to the AMOX only.



Figure (3-44) Cyclic voltammogram obtained at GOT-Nafion/ SPCE in PBS only with pH values of 2, 3, 4, 5, 6, 7, and 8 at scan rate 0.1v.s<sup>-1</sup>

The anodic peak current values of AMOX at the GOT-SPCE were plotted versus pH values of the supporting electrolyte (PBS). Fig. (3-45) revealed that the sharp increase in the oxidation peaks in the pH range of 2-3 this may be due to fast electron process then decreased in the range of 3 to 4 this can be related to change of AMOX interaction with surface of modified SPCE (GOT) [210]. Gradually increase in current was observed as the pH values increased from 4 to 6 due to oxidation ability of AMOX, where the electrochemical response of AMOX in pH7 was low. Considering all these points, the pH3 was with highest current value and show clear redox peak well than the other pH values. Therefor this value was used for the determination the limit of detections LOD.





Figure (3-45) Plots of peak current Ip for oxidation0.5mM AMOX at GOT-Nafion / SPCE *vs* pH of electrolyte solution PBS (pH3) in 0.1KCl

Cyclic voltammetry CV is a powerful tool for identifying and studying proton and electron transfer (PET). If a process involving m-protons and n-electrons as shown in equation

$$A + mH^+ + ne \rightleftharpoons B \tag{3-8}$$

For Nernst equation, assumption the electrode process was electrochemically reversible, we can write [211]

$$E = E_{f}^{\circ}(A/B) - \frac{RT}{nF} \ln [B]/[A][H^{+}]^{m}$$
(3-9)  

$$E = E_{f}^{\circ}(A/B) + RT/nFln[H^{+}]^{m} - RT/nFln[B]/[A]$$
(3-10)  

$$E = E_{f}^{\circ}(A/B) - 2.303 \frac{mRT}{nF} pH - \frac{RT}{nF} ln \frac{[B]}{[A]}$$
(3-11)

For a chemically reversible half reaction, the pH dependence of a PET couple is given by eq(3-12)[211]

$$E_{f,eff}^{\circ} = E_{f}^{\circ} \left( A/B \right) - \frac{2.303mRT}{nF} \ pH \tag{3-12}$$

 $E_{f,eff}^{\circ}$  Was an effective formal potential, the potential midway between the peaks for redox peaks for A and B. The dependence formal redox potential  $E^{\circ}$  of AMOX on the pH was investigated by cyclic voltammetry. The  $E^{\circ}$  value was obtained from the average value of anodic potential and cathodic potential. Fig. (3-46) shows that the formal potential (midpoint) of AMOX was highly pH-dependent .However, It was found that the  $E^{\circ}$  values shifted to more negative values (decrease linearly)



with increase in pH buffer solution. Between pH 2-3, a slope has value agreement with the theoretical value of 0.06V [212][213] according to the equation.

$$E^{\circ} = -0.065pH + 0.26$$

$$(3 - 13)$$

Suggesting that electrochemical oxidation of Amoxicillin involved equal number of electrons and protons [214] then the potential returend to be shifted to more positive values between pH 3-7 with a slope 0.0315 V/pH according to the equations.

 $E^{\circ} = 0.0315pH - 0.0375$ 

(3 - 14)

Sugguseted that two electrons and one proton involved in electrochemical oxidation of AMOX in the this pH range [215]. The intersection of the curve was located at pH 3 corresponding to the apparently pKa. The value of pKa is in agreement with A.T.suji et al [216] and GN. Rolinson [217]



Figure (3-46) Plots of formal potential  $E^{\circ}$  for oxidation 0.5mM AMOX at GOT-Nafion/ SPCE *vs* pH of electrolyte solution PBS ranging from (pH2-pH7) in 0.1KCl.

The cyclic voltammograms for bare SPCE, GO-SPCE and GOT-Nafion/SPCE in 0.1M blank phosphate buffer solution (pH 3.0) and in the presence of 0.5 mM AMOX was shown in Fig. (3-47).The potential was swept from - 0.5V to 0.6V at scan rate  $0.1V.s^{-1}$ . When the SPCE coated with GO (orange curve) redox peaks for AMOX were observed at 0.12 V with anodic peak current (13.186µA) and at - 0.05V with cathodic peak current (15.904µA). The cyclic voltammogram of GOT-Nafion/SPCE in blank 0.1 M phosphate buffer solution (pH 3.0) showed broad anodic and cathodic peaks (red curve). But in the presence AMOX (green curve), the anodic peak current was appeared significantly at about 0.08 V(anodic current 16.443µA), with cathodic peak on the reverse scan at -0.3V ( cathodic current 17.393µA). The increace in redox signal may be attributed to the



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functionalization of GO with 4-amino-3mercapto1, 2, 4-triazol at the edge of GO sheet which facilitate the electron movment between AMOX and the electrode surface and also could be attributed to GOT which has higher effective surface area. based on this,GOT-Nafion/SPCE can be used as electrochemical sensing for sensitive determenation of AMOX. At the bare SPCE in presence AMOX (black curve) no redox peak can be seen indicating oxidation process of AMOX hard to occur due to surface of SPCE has small amounts of oxygenated functionalities [218]. On the other hand, the bare SPCE have no electrochemical response in blank PBS (pink curve).



Figure (3-47) cyclic voltammogram of 0.5mM AMOX in 0.1M PBS (pH 3.0) at scan rate 0.1v.s<sup>-1</sup>at bare and GO-T-Nafion/SPCE.

# **3.7.2** Effect of amount of the modifier GOT-Nafion mixture on electrochemical detection AMOX

As described in experimental section (2-12) the sensor was prepared by drop a small volume of the GOT/Nafion suspension on the WE of the screen-printed electrode. CV was carried out in presence of 0.5mM of AMOX in PBS (pH 3.0) at  $0.1V.s^{-1}$  scan rate with different amounts of GOT/Nafion deposited onto the electrode surface, Fig. (3-48).





Figure (3-48) Cyclic voltammogram of amount of GOT suspension at SPCE in PBS (pH 3) containing 0.5mM AMOX .scan rate 0.1V.s<sup>-1</sup>

Many compounds have slow charge transfer rate with SPCE leading to weak defined voltammograms. Several recipes were employed to enhanced the sensitivity and electrochemical properties of carbon-based electrodes such as oxygen plasma treatment [218], preanodized[ 219], electrochemical cycling [220], these recipes enhanced the electron movement of the modified electrode (WE) through increasing activated surface sites, increasing carbon-oxygen functional (carbonyl and carboxyl) groups and remove contaminants.

This treatment made oxidation process readily occurred and increased peaks current [163]. electrochemical cycling method was used to activate the modified electrode using different volumes of modifier GOT 2, 4, 6, and 8µL by cycling the potential to extreme anodic (positive) potentials from 0 to 1.9V for 19 number of scan Figure (3-49). Using 2µL GOT suspension, no redox response found probably due to the entire surface wasn't covered, when 4µL casted, an oxidation peak current increase little. An increase in the oxidation peak current was recorded when the deposition volume was up to 6µL (this volume gets higher surface area) which also significantly increased .This volume was almost similar to literature [213]





Figure (3-49) Cyclic voltammogram for GOT-SPCE preanodized in PBS (pH3)

The oxidation peak current decreased when the amount of GOT-Nafion dispersion increased to (8 $\mu$ L), this may be due to a hindrance electron transfer result from an excessive GOT suspension [213]. From this result, the investigations will be carried out by modification SPCE with 6 $\mu$ L GOT–Nafion suspension. The surface morphology of pretreatment GOT-SPCE was characterized by SEM .From Fig. (3-50A) it is obvious the presence of pores, the surface of SPCE is smoother (nanoscale roughness) and well covered (spread uniformly) with GOT this may provide active sites (edge planes) to interact the AMOX molecules with the surface when compared with bare SPCE Fig. (3-50B)

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Figure (3-50) Scanning electron microscope (SEM) images of the surface S (A) SPCE modified with GOT. (B) bare SPCE.



#### **3.7.3 Effect of scan rate**

The effect of the potential scan rate on the electrochemical process was studied to understand the electrode behavior and the reversibility of electrode reactions. Cyclic voltammograms of GOT-Nafion/SPCE in the supporting electrolyte solution PBS (pH3) containing 0.5 mM AMOX at different scan rates from 0.01 to  $0.1V.s^{-1}$  which are shown in Fig. (3-51)



Figure (3-51) Cyclic voltammogram of 0.5mM AMOX in pH3 PBS obtained with various scan rates (0.01-0.1 V.s<sup>-1</sup>).

With increasing the scan rate, the oxidation and reduction peak current have good linear relationship with the scan rates, correlation coefficient 0.9921 and 0.9976 respectively Fig. (3-52)



Figure (3-52) Variation of the peak current  $(I_{pa})$  and  $(I_{pc})$  with the sweep rate for 0.5 mM AMOX.



The peak current is proportional to, scan rate v, surface coverage concentration  $\Gamma$  of electroactive species and number of electrons n according to the following equation. [221]

$$I_{Pa} = n^2 F^2 \, \Gamma A v / 4RT$$

(3 - 15)

A was the surface area cm<sup>2</sup>. The electroactive coverage must be high enough for a current to be observed. the surface coverage concentration of 0.1mM (AMOX) at the surface of GOT-SPCE can be estimated to be about  $5.39 \times 10^{-10}$  mol cm<sup>-2</sup> (n=2) or  $3.246 \times 10^{14}$  molecules.cm<sup>-2</sup>.

In addition, the relationship between I  $_{pa}$ , I $_{pc}$  and  $v^{1/2}$  was studied Figure (3-53). The redox peaks current were proportional to the square root of scan rate which proposed the process was a diffusion-controlled in solution [222] when transport the redox species of AMOX in solution to and from electrode.

At scan rates between 0.01 and 0.1 V/s .the peak heights of the anodic and the cathodic signals were proportional to scan rate and the ratio was almost equal. The difference of the peak potentials is significantly bigger than 59 mV and the separation of the two signals is constant at 0.35 mV  $\pm$  3 mV. Therefore, the redox reaction of AMOX can be considered to be reversible this means the electrode practically remain in equilibrium with an oxidized form of AMOX as shown in table (3-11) [ 207].

Scan rate (V.s <sup>-1</sup> )	$E_{pa}$ - $E_{pc}(V)$	I <sub>pa</sub> /I <sub>pc</sub>
0.01	0.33	1.06
0.02	0.32	0.85
0.04	0.35	1.05
0.06	0.36	1.01
0.08	0.38	1.005
0.1	0.38	0.965

Table (3-11) Peak to peak separation and peaks current ratio for AMOX at different scan rate obtained from Fig. (3-51)

The slope of the curve for the two processes, almost the same meaning that the redox process occurs with the same electron transfer rate. [223]





Figure (3-53) Relationship between  $I_{pa}$  vs. square root of scan rate for GOT modified SPCE .

The diffusion and adsorption of electrode process were studied plotted a logarithm of peaks current *vs*. logarithm of the scan rate gave a straight lines with a slope of 0.9494 for foreword scan and 0.8597for reverse scan. This value is close to that found in the literature [224] Fig. (3-54). The slope of the straight lines were intermediate value between 1 the theoretical value of the adsorption controlled electrode process and the theoretical value of 0.5 which was expressed for the diffusion controlled electrode process this indicated that the electrode process was controlled simultaneously both by diffusion and adsorption [225] for oxidation and reduction process.

The peak current arises not only of AMOX molecules which were already adsorbed on the GOT-SPCE surface but also of those which reach to the electrode by means of diffusion. These results reveal that the anodic process was dominated by partially adsorption (adsorbed AMOX at the electrode) and partially diffusion of AMOX through the diffusion layer simultaneously.





Figure (3-54) Plot of log current vs. log v

The models developed by Laviron were developed to provide a quick estimate of the electron transfer rate constant  $\kappa$  using cyclic voltammetry. This method count on the electron transfer coefficient ( $\alpha$ )( dimensionless parameter), which was a measure of the symmetry of the energy barrier of the redox reaction. To determine  $\alpha$ , the peak potential E<sub>p</sub> is plotted vs. In scan rate v [226, 227]. The peak potentials can be described by following equations.

$$E_{pa} = E^{\circ} + \frac{RT}{(1-\alpha)nF} \ln \upsilon \qquad (3-16)$$
$$E_{pc} = E^{\circ} + \frac{RT}{\alpha nF} \ln \upsilon \qquad (3-17)$$

 $E^{\circ}$  is formal potential, R is the gas constant, n was the number of electrons involved in the redox reaction, T is the absolute temperature in Kelvin, F is the Faraday constant, and  $\upsilon$  is the scan rate. The cathodic peak potential (E<sub>pc</sub>) is changed linearly as the function of scan rate in range from 0.01 to 0.1Vs<sup>-1</sup>. A linear regression equation is shown below

$$E_{pc} = -0.0277 \ln \nu + 0.4096 \tag{3-18}$$

From the slope Fig. (3-55) and taking the electron transfer coefficient 0.5 according to R.Guidelli *et al* [228] the number of electrons was estimated to be  $(n\sim2)$  this value is in agreement with value obtained from pH measurement. The E <sub>pa</sub> effort non-linear relationship .The electron transfer constant rate k<sub>et</sub> was



obtained by introducing the values  $\alpha$ , a scan rate 0.1Vs<sup>-1</sup>, and  $\Delta Ep = 0.38V$  in the following equation [229].

$$lnk_{s} = \alpha \ln(1-\alpha) + (1-\alpha)ln\alpha - ln\frac{RT}{nFv} - \frac{\alpha(1-\alpha)nF\Delta E}{RT} (3-19)$$

The rate constant can be estimated to be  $2.454 \times 10^{-3}$  cm.s<sup>-1</sup> this value indicate slow kinetics and longer time requirement for equilibrium.[226]



Figure (3-55) potential versus natural logarithm of scan rate for GOT-SPCE /AMOX.

The modified SPCE with GOT was used to estimate lower concentration of AMOX presence in solution. Using differential pulse voltammetry (DPV) technique. Fig. (3-56) showed differential pulse voltammogram under optimized conditions for varying concentrations of AMOX. The concentrations varying in the range of 1-19  $\mu$ M. The voltammogram showed that with increasing concentration of the drug the peak current linearly increased

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Figure (3-56) Differential pulse voltammograms of AMOX (PBS pH 3) at different concentrations, (down to up; 1, 4, 7, 10, 13, 16,  $19\mu$ M) scan rate 0.1 V.s<sup>-1</sup> volume of GOT suspension  $6\mu$ L.

Over concentration ranges of  $1-19 \mu M$  Fig. (3-57) the calibration curve was linear regression which can be expressed as following equation.



$$I_p(\mu A) = 0.411c(\mu M) + 9.6118$$
  $R^2 = 0.9744$  (3 - 20)

Figure (3-57) Plotting values of current versus the concentration of AMOX which plotted based the data of Fig. (3-56).



The limit of detection was calculated using the following eq. [230,231]  $LOD = 3S_{\underline{y}}/b$  (3 - 21)

Where b and  $S_{\ensuremath{y/x}}$  are the slope of the related calibration curve and standard deviation of peak current respectively

The limit of detection (LOD) for AMOX was obtained as 2  $\mu$ M. According to the obtained results, it was possible to apply this technique to the quantitative analysis of AMOX. Table (3-12) showed LOD of other method for determination AMOX.

Table (3-12) Comparison of the efficiency of some methods in the determination of AMOX.

method	pН	LOD(µM)	Reference
Adsorptive Stripping voltammetry MWCNT/GCE		0.2	[232]
Ni/Curcumin/CPE Amperometric		5	[233]
Glutaraldehyde/GlutamicAcid /GCE/SWV	5.2	0.92	[234]
SWV – MWCNT/PCE	10.5	0.0087	[235]
DPV-GOT/SPCE	3	2	This work

# **3.8 Electrochemical characterization of RGO and RGOT- SPCE with potassium ferricyanide.**

To electrochemically characterizing the fabricated modified SPCE with carbon nanomaterials RGO and RGOT, electrochemical redox probe 0.5 mM potassium ferricyanide in 0.1 KCl was used by using CV. RGO-SPCE exhibited electron-transfer reactivity towards ferri/ferro with anodic potential( $E_{pa}$ ) and cathodic ( $E_{pc}$ ) peak potential about 0.18 V and 0.04 respectively Fig.(3-58) . The anodic current (Ipa) about 9.630  $\mu$ A and cathodic peak current (Ipc) about -9.293  $\mu$ A.The redox couple gave a  $\Delta$ Ep value of 0.19 V with RGOT-SPCE lower than bare SPCE ( $\Delta$ Ep 0.24 V) and higher than RGO-SPCE ( $\Delta$ Ep 0.14 V this value is in a good agreement with literature [236].The anodic (Ipa) and cathodic (Ipc) peak current is the highest (12.358  $\mu$ A) and (12.232  $\mu$ A) respectively in RGOT-SPCE with Ipa/ Ipc=1.0103. A higher peak current and a lower peak potential of the



### Chapter three

modified SPCE electrode could provide preferable flow of electron transfer and oxidative reaction can be a more sensitivity and reactivity. Thus a presumed electrochemical response improvement could be carried out in the modification of SPCE with RGO and RGOT. It is possible to note a higher and more shaped peak after modified SPCE with RGOT than RGO. Electro catalytic activity of RGO was further enhanced by introducing the groups 4-amino,3-substituted 1H,1,2,4 Triazole 5(4H) thion or thiol (ASTT) which provided the conductive bridges to electron-transfer of ferrocyanide between the sheets. However, RGOT-SPCE modified electrodes show better results than that bare-SPCE.



Figure (3-58) CV curves of bare SPCE, RGO-SPCE and RGOT-SPCE in 0.1M KCL solution containing 0.5 mM  $K_3$  [Fe(CN)<sub>6</sub>]<sup>-3</sup>at 0.1V.s<sup>-1</sup> scan rate

### 3.8.1 Surface area study for modified SPCE with RGO and RGOT suspension

The real surface area of modified electrode RGOT-SPCE was needed [237] as the electrode reaction rate was extensive quantities. So the electrochemically active surface areas of the RGO and RGOT modified SPCE were measured. The CV of 0.5 mM K<sub>3</sub> Fe (CN)<sub>6</sub> was recorded Figs. (3-59) and (3-60).





Figure (3-59) cyclic voltammogram for RGO-SPCE in potassium ferricyanide at different scan rate.



Figure (3-60) cyclic voltammogram for RGOT-SPCE in potassium ferricyanide at different scan rates.

Under diffusion control and using Randles Sevick equation [207] at different scan rates ranging from 0.01 to  $0.1 \text{V.s}^{-1}$ . Since the value of the diffusion coefficient  $D_R$  of ferricyanide was known the value of A can be obtained, from the slope of plotting of  $I_p$  versus v<sup>1</sup>/<sub>2</sub> Fig. (3-61) and (3-62) for RGO and RGOT respectively. The electrochemically active area of bare, the modified RGO and RGOT electrodes were found to be 0.04 cm<sup>2</sup>, 0.079 cm<sup>2</sup> and 0.10076 cm<sup>2</sup> respectively .This



enhancement in surface area resulted from modified the SPCE with RGO and ROGT.



Figure (3-61) Plotting of current versus square root of scan rate for RGO-SPCE.



Figure (3-62) Plotting of current versus square root of scan rate for RGOT-SPCE.

Cyclic voltammograms of RGOT-SPCE was measured in various supporting electrolytes such as PBS, NaOH and H<sub>2</sub>SO<sub>4</sub> to show the dependence of the



electrocatalytic current of AMP .When 0.1M NaOH solution was used no obvious peak was appeared as shown in Fig. (3-62).



Figure (3-63) Cyclic voltammogram of RGOT-SPCE in AMP 0.1mM in 0.1NaOH

With  $H_2SO_4$  solution AMP showed weak oxidation peak current at 0.17V Fig. (3-64)



Figure (3-64) Cyclic voltammogram of RGOT-SPCE in AMP 0.1mM in 0.1M  $H_2SO_4$ .



However when used PBS as supporting electrolyte at different pH ranging between 2 to 10 AMP gave well define redox peaks with high peak current Fig.(3-65). At pH<sub>2</sub> the highest current value and an excellent signal enhancement Fig. (3-66) where the best deprotonation of 5-substitution 1,2,4 triazole 3-mercapto (ASTT)functional group that attached to RGO sheet.



Figure (3-65) Cyclic voltammogram of AMP response in different pH at RGOT-SPCE.



Figure (3-66) Effect of the pH on the peak current in phosphate buffers using CVat RGOT-SPCE with 0.1mM AMP concentration; scan rate,  $0.1V \text{ s}^{-1}$ 



To study the proton and electron transfer (PET) for AMP, the formal peak potential *vs.* pH at scan rate of  $0.1V \text{ s}^1$  was plotted .Fig. (3-67) showed that, there are two segments, the first corresponds to slope 0.05V/pH over the pH range of 2–5. The value of slope is close to the expected of the theoretical value of a reversible proton-coupled single electron transfer for Nernst equation [211] as shown in equation below

$$dE^{\circ}/d_{pH} = 0.0592 \ m/n \tag{3-22}$$

Suggests that the number of electrons is equal to hydrogen ions in the electrode reaction and base on this we predicated the mechanism interaction between AMP and the SPCE substrate modified with RGOT. In the second segment no obvious dependence on pH in a pH window of 6.0-9.0



Figure (3-67) Plots of formal potential, Ep for oxidation0.1mM AMP.at RGOT/Nafion modified SPCE *vs* pH of PBS (pH2) in 0.1KCl.

#### 3.8.2 Effect of drop casted volume

Voltammetric response of AMP was investigated, by drop casting different volumes of RGOT suspension on WE. Increasing the volume suspension of RGOT from 2.0 to  $4\mu$ L enhanced the peak current but at volumes more than  $4\mu$ L the peak current decrease this may be attributed to the thickness of the suspension film which blocked the surface of electrode [213]. Therefore,  $4\mu$ L was chosen as the optimum amount of RGOT suspension for fabrication of the sensor.



#### 3.8.3 Voltammetric behavior of AMP, AMOX and TET at RGOT-SPCE

The modified electrode RGOT-SPCE was examined with 0.1mM TET, AMOX as shown in Fig. (3-68) and (3-69). AMOX and TET revealed a poorly (broad) oxidation peak with low current compared with AMP Fig. (3-71), that is due to slow electron transfer. This means there was specificity for GOT-SPCE toward the AMP. So we investigated this electrochemical behavior in detailed in the following section



Figure (3-68) Cyclic voltammogram of RGOT-SPCE with TET in different pH



Figure (3-69) Cyclic voltammogram of RGOT-SPCE with AMOX in different pH.

Figure (3-70) illustrates the cyclic voltammogram of electrochemical response of AMP at bare SPCE by sweeping the potential from- 0.5V to 0.6V at scan rate 0.1V.s<sup>-1</sup>. The response of bare SPCE in presence of 0.1 mM AMP (PBS pH2) exhibits electron transfer resistance and in which AMP showed broad and poor



oxidation peak ( $0.12\mu A$ ) with a few small features on both the anodic and cathodic scans which may belong to the carbon ink itself and/or indicates low conductivity because of the existence of small amounts of oxygenated functionalities on the surface of SPCE [218].



Figure (3-70) Cyclic voltammograms of 0.1mM AMP at bare SPCE in 0.1M PBS (pH 2) and 0.1M PBS only at scan rate 0.1v.s<sup>-1</sup>.

By adding 0.1 mM AMP, a significant enhancement in the cathodic and anodic peak current was observed with RGOT-SPCE so that the modified electrode attractive for applications in the field of electrochemical sensing Fig(3-71).



Figure (3-71) cyclic voltammogram for bare, RGOT and RGO-SPCE in presence AMP in PBS pH<sub>2</sub>.



The anodic and cathodic peak potential for the oxidation and reduction of AMP at the modified SPCE with RGOT were about 0.19 V and -0.19V respectively. The RGO-SPCE didn't show any response for the AMP test, this result may be because the RGO doesn't contain enough deprotonation groups in its structure. Functionalize RGO with (ASTT) make the electrochemical redox of AMP was much stronger than RGO. However, the conductance of the prepared RGOT-SPCE displayed higher conductivity than the bare-SPCE, this may be due to the interaction between the AMP with functional group ASTT, and lead us to propose that the deprotonation induced by ASTT and the presence of the hydrogen ion affected the  $\beta$ -lactam group (Azetidine-2-one) of AMP in aqueous solution to yield the carboxylic acid group instead of ketone group, because the AMP has an electrochemically amide functional group which was as in Azetidine-2-one group. Therefore it was expected to show hydrolysis with opening of the four membered ring azetidine-2-one, in a  $H^+/1\bar{e}$  process. This redox behavior closely resembles the quinine/imine redox process in polyaniline. This case of mechanism can be applied on all antibiotic compounds which have a  $\beta$ -lactam group in its structure.

Therefore, we consider that both the deprotonation of ASTT, the interaction between the carbone materials of the screen printed electrode and the functionalized carbone nanomaterials (RGOT) and in addition to the hydrogen bonding between the  $\beta$ -lactam group and the amine groups of the ASTT facilitate better sensing performance for AMP Fig.(3-72) and (3-73).



Figure (3-72) The redox process for AMP with applied CV





Figure (3-73) The formation of carboxylic acid group instead of ketone group during the protonation process on the  $\beta$ -lactam.

Series concentrations of AMP were examined at  $pH_2$  in the range from 1 to 5  $\mu$ M using CV technique Fig. (3-74) was depending on the application of AMP the oxidation peak current was increased with the increasing of AMP concentration. At present, there was a slight shift in the peak position, it may be said that the adsorption of the side-product of the electrochemical oxidation products on the electrode surface



Figure (3-74) Cyclic voltammogram of AMP (PBS PH 2) at different con. (1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5  $\mu$ M) scan rate 0.1 V.s<sup>-1</sup> volume of RGO-T suspension 4 $\mu$ L.



Under optimal experimental conditions, the calibration plot for detecting AMP with the proposed modified electrode RGOT-SPCE was shown in Fig. (3-75). A good linear relationship between the current change and the concentration of AMP was obtained. The linear regression equation was shown below  $I_P = 1.4648c(\mu M) + 26.264$  (3 - 23) With the correlation coefficients of 0.8139. The detection limit was estimated to be 0.5  $\mu$ M. It was noted that the research is focusing on identifying and determination of the LOD of the ampicillin with the CV technique very few. For comparison, kloth et al. [238] mentioned 1.1  $\mu$ g/L for different antibiotics in raw milk, [239] et al. mentioned LOD value of 58-190 nmol/L, Khalilzadeh et al[240] mentioned 0.67  $\mu$ mol L<sup>-1</sup>by using carbon-paste electrode Modified with Ferrocendicarboxylic acid.



Figure (3-75) The relationship between current and the concentration of AMOX which Plotted with the data of Fig. (3-74)

#### 3.8.4 Effect of scan rate

Cyclic voltammograms of the RGOT-SPCE in 0.1mM AMP at different scan rate displayed evident voltammetric waves Fig. (3-76). The separation of the peak potential,  $\Delta E = E_{pa} - E_{pc}$ , increased according to the increasing of the scan rate from 0.01 to 0.1V s<sup>-1</sup> indicating that the velocity of the electron transfer is not high enough. This system is called quasi-reversible [241].





Figure (3-76) Voltammograms obtained from the RGOT-SPCE with AMP in PBS with pH= 2, varying scan rate. Con. of AMP 0.1Mm.

Plotting the anodic peak current  $I_{pa}$ , and cathodic peak current  $I_{pc}$ , against the square root of the sweep rates (v<sup>1/2</sup>) were a linear correlation at all scan rates (0.01-0.1V s<sup>-1</sup>) Figure (3-77), suggesting that the diffusion of the electroactive species on the surface of the electrode was fast to give a linear relation for *I*p against the square root of the sweep rate. The slope of the curve  $I_{pa}$  versus square root of scan rate is greater than that corresponding to the redaction process, meaning that the oxidation process occurs with a higher electron transfer rate and the current depends on the concentration of AMP which is oxidized as soon as it reaches the surface of the RGOT-SPCE [224].





Figure (3-77) Linear relationship between Ip *vs*. square root of scan rate for AMP at RGOT-SPCE.

As shown in table(3-13), the peak heights of the anodic signal and the cathodic signal are not completely equal when the scan rate varies between 0.01-0.1V.s<sup>-1</sup> indicated that the process is not completely reversible[242] anymore Also the difference of the peak potentials was significantly bigger than 59 mV and the separation of the two signals increases with faster scan rates revealing that electron transfer kinetics of AMP at higher scan rates can be considered to be quasireversible behavior [242].

Table (3-13) Potential separation  $\Delta E_p$  with ratio of anodic and cathodic peaks current for RGOT-SPCE in presence AMP at different scan rate

Scan rate (V.s <sup>-1</sup> )	$E_{pa}$ - $E_{pc}(V)$	I <sub>pa</sub> /I <sub>pc</sub>
0.01	0.26	1.26
0.02	0.29	1.12
0.04	0.34	1.28
0.06	0.33	1.25
0.08	0.42	1.44
0.1	0.46	1.71



Furthermore, from the slope of the linear plot of  $I_{Pa}$  vs. scan rate for RGOT-SPCE and bare SPCE Fig. (3-78) and Fig. (3-79) respectively, the surface coverage concentration  $\Gamma$  of the electroactive species 0.1mM (AMP) that adsorbed at the surface of RGOT-SPCE and bare SPCE can be estimated(using eq.3-15) to be about  $6.73 \times 10^{-10}$  molcm<sup>-2</sup> and  $3.37 \times 10^{-11}$  molcm<sup>-2</sup> (n=1) respectively.

This high surface concentration for RGOT-SPCE was attributed to the functionalized RGOT deposited in SPCE this means there is strong interaction of AMP to modify electrode than bare SPCE. According to equation (3-18)



Figure (3-78) Variation of the peak current  $(I_{pa})$  and  $(I_{pc})$  with the sweep rate for 0.1mM AMP at RGOT-SPCE



Figure (3-79) Variation of the peak current ( $I_{pa}$ ) with the sweep rate for 0.1mM AMP at bare SPCE



A plot of logarithm the peaks of current *vs.* logarithm of scan rate gave a straight line with a slope of 0.833 for foreword scan and 0.718 for reverse scan Figure (3-80). The slope of the straight lines was intermediate value between 1 the theoretical value of the adsorption controlled electrode process and the theoretical value of 0.5 which is expressed for the diffusion controlled electrode process, this indicated that the electrode process was controlled simultaneously both by diffusion and adsorption [226] and depended on the scan rate [243].



Figure (3-80) Plot of log current vs. log scan rate v (V/s).

The electron-transfer coefficient ( $\alpha$ ) and electron-transfer rate constant k can be easily determined according to Laviron's equations [227]:

$$E_{pa} = E^{\circ} + \frac{RI}{(1-\alpha)nF} \ln \upsilon \qquad (3-24)$$
$$E_{pc} = E^{\circ} + \frac{RT}{\alpha nF} \ln \upsilon \qquad (3-25)$$

Fig. (3-81) showed the relationship between the peaks potential (Ep) and the natural logarithm of the scan rate of the RGOT-SPCE. The cathodic peak potential ( $E_{pc}$ ) and anodic peak potential ( $E_{pa}$ ), in the range from 0.01 to 0.1Vs<sup>-1</sup>, was changed linearly as the function of scan rate with a linear regression equation of y =-0.0543x-0.035 and y=0.0302x+0.0124 respectively. The slope for cathodic peak equals to  $-RT/\alpha nF$  and for anodic peak equals to  $RT/(1-\alpha) nF$  . $\alpha n$  can be calculated as 0.47. According to Ref. [244], it is usual that the value of  $\alpha$  is 0.3 <  $\alpha$  < 0.7. Thus, with n = 1 the value of  $\alpha$  will be 0.47. This indicates that the redox reaction between AMP and RGOT-SPCE was a single electron transfer process. This result corresponds with value predicated from pH measurement. The value of apparent



heterogeneous electron transfer rate constant ( $k_s$ ) could be calculated using Eq. (3-25) [244].

$$lnk_s = \alpha \ln(1-\alpha) + (1-\alpha)\ln\alpha - \ln\frac{RT}{nFv} - \frac{\alpha(1-\alpha)nF\Delta E}{RT} (3-26)$$

The value of k<sub>s</sub> is calculated as  $5.4 \times 10^{-2}$  .RGOT-SPCE showed a faster electron transfer rate, having the higher slopes of 105.41 µA (V. s<sup>-1</sup>)<sup>-1/2</sup> compared with RGOTM-SPCE of 32.92 µA (V. s<sup>-1</sup>)<sup>-1/2</sup> which have value of k<sub>s</sub> about  $4.2 \times 10^{-3}$ .



Figure (3-81) Plot of variation of peaks potential with natural logarithm of scan rate for AMP at RGOT-SPCE.

#### 3.8.5 Stability

The stability of RGOT-SPCE was investigated during storage in dray situation in 25°C shown in Fig. (3-82), by cycling the potential forty times of CV measurement at RGOT-SPCE in 0.1Mm AMP in 0.1M PBS (pH2). The peak current intensities, I <sub>pa</sub> and I <sub>pc</sub>, measured between the potentials – 0.5 and 0.6V at scan rate of 0.1V s<sup>-1</sup>. the RGOT-SPCE offers good measurement stability. Little decrease of peaks current was observed with increase of scanning cycle. The cathodic peaks current hold 90% of its initial current after 10days. The electrode can be considered to have a good chemical stability, allowing uses it without significant variation in response.





Figure (3-82) Cyclic voltammogram (40cycle) of 0.1mM AMP in PBS (pH2) at RGOT-SPCE, scan rate 0.1V.s<sup>-1</sup>

#### 3.8.6 Surface characterization of modified SPCE with RGOT

The WE surface of the SPCE play a very important role, because the area of the electrode was one of the major causes to increase or decrease the efficiency of the sensor and its results. The coated surface of the WE with nanomaterial (RGOT) increase the total surface and added the new properties of the nanomaterial. Fig. (3-83) shows SEM image of bare and modified WE with (RGOT) in microscale (A and B) and in nanoscale (C and D) respectively. The images show that the surface was slightly rough and disordered in bare-SPCE Fig.(3-83C), while modified electrode with RGOT seems to be an overall smoother than the bare-SPCE. However, the granule sizes appeared smaller than for the bare electrode on the existence of accumulated RGOT sheets.





Figure (3-83) Scanning electron microscope (SEM) images of the surface details. The SEM micrograph of bare and modified SPCE are shown A-D

#### 3.8.7 Surface characteristics by AFM measurement

The characterization of the bare and modified WE surface with RGT was achieved by measuring the roughness parameters extracted from AFM topography using PHYWE software. Quantitative morphological characteristics such as peak height Rp, peak-valley height Ry, valley depth Rv average roughness (Ra), and root mean square roughness (Rq) were obtained and tabulated in table (3-14). Nanoscale surface features were visualized as shown by 3D AFM images in Fig.


(3-84), (A) represented the bare SPCE and (B) the modified SPCE respectively. The modified SPCE has lower values for all parameters than bare SPCE.

The values in table (3-14)show a decrease in the values of Rp , Ry, Rv, Ra and Rq for RGOT-SPCE, these results occur through the filling of the valley depth with RGOT nanomaterials and this reduced the valley height and the average roughness became smaller, this indicated to a well distribution of the carbone nanomaterials (RGOT-SPCE) within the carbone surface of the bare SPCE and came to an agreement with the SEM images results Fig.(3-83) this good distribution was one of many factors to create an accurate measurement data.

parameter	Bare SPCE	Modified SPCE
peak height Rp	47.817 nm	11.835 nm
peak-valley height Ry	84.272 nm	34.602 nm
valley depth Rv	-36.455 nm	-22.767 nm
average roughness (Ra)	12.921 nm	4.9568 nm
root mean square roughness (Rq)	16.592 nm	6.0457 nm

Table (3-14) Surface parameter for bare and modified SPCE with RGOT

_	<u> </u>
β	124
lŀ	



Figure (3-84) 2D and 3D AFM images for (A) bare electrode (B) modified WE with RGOT nanomaterial

# **3.9 Electrochemical characterization of RGOTM-SPCE using ferricyanide as probe.**

Fig. (3-85) shows the electrochemical response of 0.5mM K<sub>3</sub>Fe (CN) <sub>6</sub> in 0.1 KCl, this redox pair is very sensitive to the state of the surface and has been used in the investigations of electrocatalytic activity of carbon electrodes [245]. A potential window, the ranges were selected from -0.3V to + 0.6V. At bare SPCE well defined redox peaks were observed and the peak-to-peak separation  $\Delta Ep$  was 0.24 with the small redox peak current. When the RGOTM solution immobilize on SPCE a pair of redox peak current increased clearly than bare SPCE and  $\Delta Ep$  value decrease to 0.22 V ,indicating that RGOTM increased the electron transfer



rate of potassium ferricyanide. Also the anodic ( $I_{pa}$ ) and cathodic ( $I_{pc}$ ) peak current is highest with Ipa/ Ipc=1.08 this value was identical for simple reversible couple. In light of the above results, the fastest electron transfer electrochemical reaction occurs at RGOTM-SPCE than the bare SPCE. We found the  $\Delta$ Ep value for RGOT lower than RGOTM suggesting that the charge transfer resistance was a little less than RGOTM.



Figure (3-85) Cyclic voltammogram of RGOTM and bare SPCE in presence 0.5mM K<sub>3</sub>Fe (CN)  $_6$  in 0.1 KCl

#### 3.9.1 Surface area study

Surface area study shows a clear evidence for the effective modification of Bare SPCE with modifier GOTM, 0.5 mM  $K_3Fe(CN)_6$  was used to measure the effective surface areas of both bare and modified SPCE by CV at different scan rates Fig.(3-86)





Figure (3-86) cyclic voltammogram for RGOTM-SPCE in potassium ferricyanide.

In the reversible system, the peaks current should follow Randles Sevick equation [207]. The areas of bare SPCE and modified RGOTM-SPCE were estimated from the slope of peaks Ip ( $\mu$ A) vs square root of scan rate v<sup>1/2</sup> V.s<sup>-1</sup> Fig. (3-87) and found to be 0.04 cm<sup>2</sup> and 0.0768 cm<sup>2</sup> respectively. Thus, there was an enhancement in the effective surface area when SPCE was modified with RGOTM, which is a strong evidence for the successful and effective modification of SPCE using RGOTM.



Figure (3-87) plotting of current versus square root of scan rate RGOTM-SPCE.



#### 3.9.2 Voltammetric behavior of AMP, AMOX and TET at RGOTM-SPCE.

The influence of pH on the oxidation peak current and potential of AMP, AMOX and TET in PBS pH range (2 to 10) at the GOTM-Nafion modified SPCE was investigated by CV at scan rate  $0.1V.s^{-1}$ . Fig. (3-88) showed the cyclic voltammogram for AMOX, the peak anodic current was broad. The peaks current that started to generate at potential lower 0.4 V with peaks around 0.6 v may belong to the presence of MnO<sub>2</sub>. The modified SPCE with RGOTM doesn't show any response with TET as shown in Fig. (3-89)



Figure (3-88) Cyclic voltammogram of RGOTM –SPCE with AMOX in different pH.



Figure (3-89) Cyclic voltammogram of RGOTM –SPCE with TET in different pH.

Fig. (3-90) showed existence of well-defined a pair of redox peaks of AMP. Greater current response is observed at pH 2 as shown in Fig. (3-91)





Figure (3-90) cyclic voltammogram of RGOTM -SPCE/AMP in different pH





The formal peak potential *vs*. pH was plotted at scan rate of  $0.1 \text{V s}^1$  Fig. (3-92) showed that, there were two segments, the two segments correspond to slope 0.01 V/pH over the pH ranges, suggesting that electron transfer is not accompanied with a proton transfer





Figure (3-92) Plots of peak potential, Ep for oxidation 0.1mM AMP.at RGOTM-Nafion modified SPCE *vs* pH of PBS in 0.1KCl.

### 3.9.3 Electrochemical behavior of APM at RGOTM /Nafion-SPCE

The cyclic voltammograms for RGOTM/Nafion-SPCE in 0.1M blank phosphate buffer solution (pH 2) and in the presence of 0.1m M AMP by sweeping the potential from - 0.3V to 0.6V at scan rate 0.1V.s<sup>-1</sup> were shown in Figure(3-93). The response of RGOTM/Nafion-SPCE showed in presence of PBS (pH2) two anodic and cathodic peaks current with height about 7.2  $\mu$ A and -5.577  $\mu$ A respectively. This redox couple was associated with modified SPCE with RGOTM. The test of AMP show the anodic peak current (height 13.98  $\mu$ A) and significantly appeared at about 0.13 V, with cathodic peak (height - 13.198 $\mu$ A) on the reverse scan at -0.21 V( $\Delta E_p = 0.34$  V). this increace in electrochemical signal come from precence of AMP.





Figure (3-93) cv for RGOTM-SPCE in presence AMP in PBS  $pH_2$  and in PBS  $pH_2$ 

Scan rate studies were carried out to appreciate whether the processes at the GOTM modified SPCE was under diffusion or adsorption controlled therefore, the electrochemical behaviors of AMP at different scan rates were investigated with CV Fig.(3-94)



Figure (3-94) Voltammograms obtained from the RGOTM-SPCE/AMP in PBS with pH 2, with varying scan rate. Concentration of AMP 0.1mM, at 25°C.



A linear relationship was observed between the peak current and the scan rate in the range 0.1 to 0.1V.S<sup>-1</sup> Figure (3-95). The regression equation obtained from CV analysis is:



Figure (3-95) Variation of the peak current  $(I_{pa})$  and  $(I_{pc})$  with the sweep rate for 0.1mM AMP.

The peak heights for the anodic and the cathodic signal are almost equal. Also, the potential difference between the anodic and cathodic potential is almost constant at  $0.36V \pm 2 V$  at scan rates between 0.01 and  $0.1V \cdot s^{-1}$ .so AMP fulfills the criteria of a reversible reaction [223] as shown in table (3-15).

Table (3-15) Potential separation  $\Delta E_p$  with ratio of anodic and cathodic peaks current for RGOTM-SPCE in presence of AMP at different scan rates.

Scan rate (V.s <sup>-1</sup> )	$E_{pa}$ - $E_{pc}(V)$	I <sub>pa</sub> /I <sub>pc</sub>
0.01	0.35	1.2
0.02	0.35	0.96
0.04	0.36	0.95
0.06	0.37	1.03
0.08	0.38	1.00
0.1	0.35	1.06

The surface coverage concentration  $\Gamma$  of 0.1mM (AMP) that adsorbed at the surface of RGOTM-SPCE was estimated from slope to be  $3.04 \times 10^{-10}$ .also a linear dependence of the peak current upon the square root of the scan rate was found Fig. (3-96), demonstrating a diffusional behavior that is, the rate of electron



transfer is controlled by diffusion the redox species (AMP) from the bulk to the electrode [246].



Figure (3-96) Linear relationship between I pa of AMP vs. square root of scan rate.

The regression equation obtained from CV analysis is  

$$I_{pa} = 32.921v^{1/2} + 2.6347$$
  $R^2 = 0.946$  (3 - 29)  
 $I_{pc} = -33.37v^{\frac{1}{2}} - 2.2394$   $R^2 = 0.9872$  (3 - 30)

The slope of the curve  $I_p$  versus square root of scan rate were almost similar in oxidation and reduction process, meaning that the redox process occurs with the same electron transfer rate. A plot of logarithm the peaks of current *vs*. logarithm of scan rate showed a straight line with a slope of 0.3153 for foreword scan and 0.3594 for reverse scan Fig. (3-97).



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Figure (3-97) Plot of log current vs. log scan rate v (V/s).

The slope of the straight lines was close to the theoretically expected value of 0.50 for a diffusion controlled process [226]. Therefore, the electrode process has a diffusion control without any adsorption phenomenon complicating the electron transfer. Transfer coefficient  $\alpha$ n can be determined from the slope of the plot of the peak potential of a cyclic voltammogram against the natural logarithm of the scan rate, v, where slope is equal to the following

$$slop = RT/2\alpha_a nF$$

(3 - 31)

This equation hold for reacting species that diffuses toward the electrode without being specifically adsorbed [229].the linear regression of relation above was y = 0.0136x + 0.154(3 - 32)From slope  $\alpha n$  can be estimated as 0.94. Substituted value of  $\alpha$  0.5[229]. Number of electron can be found as 2. The  $E_{pc}$  effort non-linear relationship. The value of apparent heterogeneous electron transfer rate constant (ks) could be calculated using Eq. (3-25). The value of k<sub>s</sub> calculated as  $4.2 \times 10^{-3}$ 

### **3.9.4 SEM characterization**

The surface morphology of modified working electrode WE with RGOTM was studied by SEM, from Fig. (3-98) it were clear the presence of RGOT sheets with particles of MnO<sub>2</sub> as they were shown in oval and circle, A represented the carbone substrate of the SPCE, where B represented the RGO mono layer sheet and spread the MnO<sub>2</sub> nanoparticles which appeared in the oval and circle on it



Figure (3-98) SEM image of WE modified with RGOTM



# **3.10** Electrochemical characterization of GOTM -SPCE Electrode using ferrocyanide.

C-V is an effective and convenient technique for probing the feature of the modified electrode surface. Here in, C-V was employed to investigate the electrochemical properties of GOTM-SPCE electrode .CV experiment have been conducted in 0.1M KCl solution containing 0.5mM  $K_3$ [Fe(CN)<sub>6</sub>]<sup>-3</sup>at 0.1V.s<sup>-1</sup> scan rate Fig.(3-99).



Figure (3-99) cyclic voltammogram of bare and GOTM/Nafion-SPCE in0.8mM potassium ferricyanide.

High value of redox peaks current was obtained for GOTM/Nafion-SPCE compared with bare SPCE. The cyclic voltammogram of K<sub>3</sub>Fe (CN)<sub>6</sub> at bare SPCE has low current signal [ $\Delta$ Ep for bare SPCE was 0.24 V]. However, the voltammetric response was apparently improved at GOTM/Nafion-SPCE reflected by the enlargement of the peak currents (Ip) and the ramp of the potential difference [ $\Delta$ Ep GOTM/Nafion-SPCE is 0.23V] between the reduction and the oxidation peaks curve. The ratio of the anodic and cathodic peak currents reached unity (*I*pa/*I*pc = 1.02), indicating that GOTM could promote electron transfer of [Fe (CN)<sub>6</sub>]<sup>-3/-4</sup> quite well due to its high surface area

#### 3.10.1 Surface area study

The microscopic areas of the bare SPCE and GOTM/Nafion-SPCE was measured. Cyclic voltammograms were recorded at different scan rates for GOTM/Nafion-SPCE in presence of 0.5 mM K<sub>3</sub> [Fe (CN)<sub>6</sub>]<sup>-3</sup>Fig. (3-100)





Figure (3-100) Cyclic voltammogram for GOTM-SPCE in potassium ferricyanide

The obtained current were plotted against the square root of scan rates in case Figure **GOTM-SPCE** (3-101).The regression equations obtained for GOTM/Nafion-SPCE were

$I_{na}(\mu A) = 19.321\nu^{\frac{1}{2}} + 1.0876$	$R^2 = 0.9861$	(3 - 33)	
$I_{nc}(\mu A) = -23.498\nu^{\frac{1}{2}} - 0.3677$	$R^2 = 0.9975$	(3 – 34)	
Randles-Sevcik equation [207] was	used to measure t	he effective surface area	of
bare and modified electrode from	the slope of the	straight line. There was	a
difference in values of the slopes	for both anodic	and cathodic currents f	or
ferricyanide in GOTM/Nafion-SPCE	E indicated a diffe	erence in the diffusion ra	ate
[224]. The effective surface area incr	reased to 0.05 cm <sup>2</sup>	when SPCE modified with	ith
GOTM. This enhancement in surface	area was resulted	in the remarkable cataly	tic

activity of the modified electrode.





Figure (3-101) Plotting of current versus square root of scan rate for GOTM-SPCE in presence of 0.5 mM  $K_3$ Fe (CN) <sub>6</sub> at scan rate 0.1V/s

### **3.10.2** Voltammetric behavior of Tetracycline at GOTM–SPCE

Fig. (3-102) illustrated the cyclic voltammogram of electrochemical response of TET at the GOTM-SPCE electrode and bare SPCE by sweeping the potential from- 0.6V to 0.8V at scan rate 0.1V.s<sup>-1</sup>. The response of bare SPCE in presence of 0.1 mM TET (PBS pH2) exhibits electron transfer resistance and in which TET showed broad and poor oxidation peak with a few small features on both the anodic and cathodic scans, which may be due to the carbon materials itself



Figure (3-102) cyclic voltammograms of 0.1mM TET at bare SPCE in 0.1M PBS (pH 2) and 0.1M PBS only at scan rate 0.1v.s<sup>-1</sup>.



From slope of plotting current versus different scan rates Fig.(3-103) The surface coverage concentration ( $\Gamma$ ) of TET at bare –SPCE was found by using eq.(3-15) [221]As 3.979×10<sup>-12</sup>mole.cm<sup>-2</sup> or 26.26×10<sup>11</sup>molecules.cm<sup>-2</sup>



Figure (3-103) linear relationship between I  $_{pa}$  vs. scan rate v V.s<sup>-1</sup>for bare-SPCE in presence of 0.1mM TET.

Fig. (3-104) was demonstrated the cyclic voltammogram of GOTM-SPCE and bare SPCE in presence of 0.1mM of TET in PBS (pH2) and PBS (pH2) only. The voltammogram exhibited a well-defined two anodic peaks at  $E_p^{a1}$ =0.698V and  $E_p^{a2}$ = 0.08V with a pair of cathodic peaks  $E_p^{c1}$ = -0.2197V and  $E_p^{c2}$ = -0.3841V. The features that are present in bare electrode tend to be dwarfed in modified SPCE. An oxidation peak was appeared at 0.698 V corresponding to presence of GOTM on SPCE. This oxidation peak confirmed presence of MnO<sub>2</sub> on GOT sheet as reported in literature [247]. Cathodic peak at -0.2197 v was assigned to the MnO<sub>2</sub> reduction [248] as shown in the following equation

$$MnO_2 + H^+ + e^- \to MnOOH \tag{3-35}$$

The other two redox peaks were assigned to oxidation and reduction of TET at GOTM-SPCE, as can be seen the cathodic reduction peak was not distinctly visible (much broader). The anodic peak current clearly increased compared with bare SPCE this may be attributed to MnO<sub>2</sub> nanoparticles which have catalytic properties for oxidation TET. In addition GO functionalized with TCH may enhanced catalytic activity through synergies with MnO<sub>2</sub> nanoparticles all this could accelerate the transfer rate of electron.





Figure (3-104) Cyclic voltammograms of electrochemical response of TET at the GOTM -SPCE and bare SPCE at scan rate 0.1V.s<sup>-1</sup>

#### 3.10.3 Effect of amount of the GOTM-Nafion dispersion

The amount of modifier compounds related to voltammetric response, so the relation between the volume of suspension (GOTM/nafion) and the redox peaks current was examined Fig.(3-105) when the amount of GOTM suspension is 2µL no change in current values would be detected (a very thin film). In addition, the higher current is obtained when amount of modifier increases to  $4\mu L$  (high surface area). With further increase in the amount of GO-TM-Nafion dispersion, the oxidation peak current showed a drastic decrease, this indicates the hindrance to the electron transfer of TET due to excessive GOTM [214].



Figure (3-105) cyclic voltammogram of amount of suspension GOTM at SPCE in PBS (pH 2) containing 0.1mM TET .scan rate 0.1V.s<sup>-1</sup>



#### 3.10.4 Effect of pH

The influence of pH value was studied to show electrochemical response of 0.1 mM TET with GOTM modified electrode with CV technique. Fig. (3-106) showed the effect of pH in 0.1 mM TET in buffer solution (PBS) ranging from pH2 to 8. With increasing the pH values of the solution, the cathodic peaks were disappeared of  $MnO_2$ . Also anodic peaks current corresponding to the oxidation of  $MnO_2$ , decreased progressively with increasing pH solution.



Figure (3-106) Cyclic voltammogram obtained from presence 0.1  $\mu$ M TET with modified SPCE with GOTM at varying pH of PBS (pH2to pH8) at scan rate 0.1Vs<sup>-1</sup>.

The oxidation peak current of TET was decreased significantly from pH2to pH8 as shown in Fig. (3-107) .At pH 2, an excellent signal enhancement accompanied by greater current response was obtained. Thus, in subsequent work, a supporting electrolyte PB of pH 2.0 was chosen.



Figure (3-107) plot of  $I_{Pa}$  vs. pH for cyclic voltammogram of 0.1µM TET in PBS at pH ranging from 2 to 8



The high peak current at pH2 may be attributed to the TET in high acid conditions undergoes dehydration to yield anhydrotetracycline [249] [250], as shown in equation below:



The possible oxidation sites are present in anhydrotetracyclin molecule hydroxide groups, carbonyl and amide. In addition the electrochemical reduction of  $MnO_2$  (introduction of protons and electrons in its structure) [251] makes TET oxidized and gives high peak current. The disappearing of oxidation peak of TET with increasing pH to 8 may be due to transform TET to isotetracycline [252]. From this behavior, it was possible to deduce, that the anodic peak current of TET attributed to the hydroxyl group not to amide group.



#### **3.10.5 Effect of scan rate**

The effect of varying potential scan rate on the peak potential and peak current of TET was studied by CV in the scan rate range 0.02-0.1 V.s<sup>-1</sup> Fig. (3-108)





Figure (3-108) Cyclic voltammogram of 0.1mM TET in pH2 PBS obtained with various scan rate (0.02-0.1 V.s<sup>-1</sup>).

The peaks at potentials(> - 0.2 V) and 0.6 V belong to redox of MnO<sub>2</sub>. There was no corresponding reduction peaks observed at the reverse scan, indicating that the electrochemical oxidation of TET were totally irreversible reaction. This may be due to that oxidized form of TET will be converted to Product as in the case of an *E C irr* mechanism *and* the rate constant k of reaction (3-37) is finite. I<sub>pc</sub> wasn't appeared at slow and fast scan rates. This means the constant rate reaction for oxidized form was large (the chemical reaction step might be relatively faster than the electron transfer for the oxidation reaction)

 $Estep \qquad R \leftrightarrow OX + ne^- \qquad (3-36)$ 

Cstep  $OX \leftrightarrow P$  (3-37) By increasing the scan rate, the oxidation peak current show

By increasing the scan rate, the oxidation peak current shows a good linear relationship Fig. (3-109) The average surface coverage ( $\Gamma$ ) of TET on the surface of modified electrode GOTM could be estimated based on the slope of current I<sub>p</sub> vs. scan rate v [253].the surface concentration of TET at GOTM-SPCE was found to be  $1.60 \times 10^{-9}$  mole cm<sup>-2</sup> or  $10.56 \times 10^{14}$  molecule cm<sup>-2</sup> by the involvement of two electrons in the process. This high surface concentration (higher than bare) can be attributed to modify the electrode with GOTM nanomaterial. The larger surface area of the GOTM nanomaterial may be helpful for more of TET molecules to take part in the electron transfer process.





Figure (3-109) linear relationship between I  $_{pa}$  vs. Scan rate v V.s<sup>-1</sup>for GOTM-SPCE in presence 0.1mM TET

The dependence of the oxidation peak current  $I_p^{ox}$ , on the square root of the scan rate  $v^{1/2}$  at the GOTM electrode was presented in Fig. (3-110). Anodic peak current at the GOTM electrode increases linearly with  $v^{1/2}$  indicating a diffusion-controlled process in solution, according to the Randles–Ševčik equation.



Figure (3-110) Linear relationship between I  $_{pa}$  vs. square root of scan rate v V.s<sup>-1</sup>.



The plotting of log-log peak current versus scan rate considers a primary quantitative to distinguish simple adsorption peaks from diffusion peaks. However, adsorption and diffusion peaks may be overlapped and difficult to distinguish. A plot of log ( $I_p \mu A$ ) vs log (v Vs<sup>-1</sup>) Fig.(3-111) gave straight line with a slope of 1.002 correlation coefficient 0.9927. The slope of the straight lines was similar to the theoretical value of the adsorption controlled electrode process [226].



Figure (3-111) Plot of log current vs. log scan rate v

This means the TET molecules that were adsorbed at the electrode will undergo electron transfer process [226].and has significant interaction with the electrode surface Fig. (3-112)



Figure (3-112) Electrochemical mechanism that involved adsorbed species.

Additionally, the peak potential  $E_{pa}$  of oxidation TET gradually shifted to the positive direction with increasing the scan rates Fig. (3-113) which confirms the irreversibility of the process [254].





Figure (3-113). Linear relationship between E (V) vs. Scan rate v (V.s<sup>-1</sup>)

The  $E_p$  of the oxidation peak was also dependent on scan rate Fig. (3-114) shows the relationship between the peak potential ( $E_p$ ) and the logarithm of scan rate (logv) for the modified electrode GOTM in 0.1 mM TET. As can be seen, in the range from 20 to 100 mV/s, the anodic peak potential ( $E_{pa}$ ) changed linearly versus *logv* which is expressed by the equation

$$E_n(V) = 0.045 \log v (mV S^{-1}) + 0.0113 \qquad (3-38)$$







For an adsorption-controlled and irreversible electrode process, Ep was defined according to Laviron equation [255,256]

$$E_p = E^{\circ} + \left(\frac{2.303RT}{\alpha nF}\right) \log\left(\frac{RT\kappa^{\circ}}{\alpha nF}\right) + \left(\frac{2.303RT}{\alpha nF}\right) \log \upsilon \qquad (3-39)$$

The slope of the linear regression of Ep vs. logv Fig. (3-114) was equal to  $2.303RT/\alpha nF$ . The evaluated value of  $\alpha n$  can be calculated to be 1.3111.  $\alpha$  was assumed as 0.5 for a totally irreversible electron transfer, it could be concluded that  $n \cong 2$ . The value of  $\kappa^{\circ}$  was determined from the intercept of the above plot if the value of E° was known. The value of E° can be obtained from the intercept of Ep vs v curve Fig. (78) By extrapolating to the vertical axis at v=0 [257]. E° can be obtained to be 0.0425 V.

So the apparent electrochemical rate constant  $k^{\circ}$  was calculated to be 10.41 s<sup>-1</sup>.

#### 3.10.6 Calibration curve and detection limit

Differential pulsed voltammetry (DPV) was employed as a technique for the evaluation of the performance of the GOTM-SPCE sensor for the determination of TET concentration under the optimized experimental conditions. The increasing of TET concentration on the WE increased the peak current linearly and shows a little change in the potential values with DPV measurements Fig. (115).



Figure (3-115) Differential pulse voltammograms at a GOTM-SPCE for TET concentrations ranging  $1-9\mu M$ .



The calibration curve was linear over concentration ranges of 1–9  $\mu$ M Fig. (3-116).The linear regression equations can be expressed as



$$(3-40)$$



Figure (3-116) calibration curve that depicts the lowest level at which TET detection is done

The LOD was determined to be  $0.2 \times 10^{-6}$  M. The TET detection in other ways is showen in Table (3-16).

Used method for detection	LOD (µM)	References
Fluorescence optical fiber sensor	0.106	[258]
Molecularly Imprinted Technique	0.0558	[259]
high-throughput suspension array technology	0.00034	[260]
high pressure liquid chromatographic	0.021	[261]
aptasensor	0.0003	[262]
Electrochemical sensor	0.2	This work

The modified electrode GOTM-Nafion /SPCE also was examined with AMP and AMOX. There was no redox peaks for AMP or AMOX in this modified electrode even after cleaning the electrode surface in phosphate buffer (in absence of the target analyte) by high anodic (+2V) potential. The peaks current at around 0.6 V belonged to  $MnO_2$  that immobilized at GOT sheet as shown in Figs. (3-117 and 3-



118) respectively. This means there was specificity for GOTM-Nafion /SPCE toward the TET.



Figure(3-117) Cyclic voltammogram of 0.1mM AMP in 0.1M PBS (pH2) at scan rate 0.1v.s<sup>-1</sup>at GOTM-Nafion/SPCE



Figure (3-118) Cyclic voltammogram of 0.1mM AMOX in 0.1M PBS (pH 2) at scan rate 0.1v.s<sup>-1</sup>at GOTM-Nafion/SPCE.



### 3.11 Electrochemical properties of (f-MWCNT) and its nanocomposites.

Carbon nanotubes (CNTs) have received great attention especially in the field of sensors due to their unique properties. In this section the electrocatalytic properties of f-MWCNT and its composite with GOT were studied.

#### 3.11.1 Surface area study.

Drops casted method was used to study voltammetric response of modified SPCE with different volumes of MWCNT without treatment, MWCNT with treatment (f-MWCNT) and its nanocomposites with GOT (f- MWCNT-GOT) using ferri/ferro redox couple system. Fig (3-119) showed the modifier f-MWCNT and f-MWCNT-GOT nanocomposites exhibit higher current response in presence of ferric/ferro couple and can be use more volumes ( $18\mu$ L) and ( $14\mu$ L) respectively compared with plain MWCNT ( $2\mu$ L) Also response of f-MWCNT/GOT didn't change. This enhanced in heterogeneous electron transfer rate for f-MWCNT is due to presence of the ASTT groups functionalized attached at the edge of GO sheets in nanocomposite accelerate electron transfer between ferro/ferric and electrode surface. The higher amount of f-MWCNT and its nanocomposite didn't make the film removed from the electrode surface.



Figure (3-119)Volume of MWCNT, f-MWCNT and f-MWCNT-GOT from cyclic voltammogram of modified SPCE in 0.8 mM Potassium ferricyanide in 0.1M KCl



The Randles Sevick equation [207] was used to estimate effective surface area of the modified SPCE with MWCNT, f-MWCNT and f-MWCNT-GOT using 0.5mM potassium ferrocyanide as probe. Cyclic voltammetric measurements were performed at various scan rates ranging from 0.03 to 0.18V.s<sup>-1</sup>.by plotting Ip versus square root of scan rates fig. (3-120), (3-121) and (3-122) surface of MWCNT-SPCE, f-MWCNT-SPCE and f-MWCNT-GOT-SPCE was estimated as 0.04, 0.119 and 0.12 respectively.



Figure (3-120). Linear relationship between I pa vs. square root of scan rate for MWCNT-SPCE



Figure (3-121) Linear relationship between Ipa vs. square root of scan rate for f-MWCNT-SPCE.





Figure (3-122) Linear relationship between  $I_{pa}$  vs. square root of scan rate for f-MWCNT-GOT.

According to method by Sharp [221]eq.(3-15) and from the slope of the anodic peak currents *versus* various scan rate as shown Fig. (3-123) an approximate of the surface coverage  $\Gamma$  (mol cm<sup>-2</sup>) of the modified electrode was estimated for MWCNT-SPCE and f-MWCNT-GOT/SPCE and found as 9.447×10<sup>-9</sup>, 9.449×10<sup>-10</sup> and 9.3×10<sup>-10</sup> mol cm<sup>-2</sup>, for MWCNT, f-MWCNT and f-MWCNT-GOT respectively, The surface coverage concentration for MWCNT and f-MWCNT are almost the same, this means the electron transfer was facilitated by functionalization of MWCNT with carboxylic groups not by surface area as the surface coverage was the same for both for nanocomposite. Also The values of surface coverage concentration  $\Gamma$  for SPCE modified with MWCNT lower than other electrodes due to absence of functionalities.





Figure (3-123) Variation of the peak current  $(I_{pa})$  and  $(I_{pc})$  with the sweep rate for modified SPCE MWCNT, f-MWCNT and f-MWCNT-GOT in 0.8mM potassium ferrocyanide.

The modified electrode is electrochemically tested to investigate its electroactivity and to improve it for a variety of applications. The standard solution of  $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$  couple was used for comparing the voltammetric behavior of modified screen-printed electrodes. This redox couple shows a simple and well-defined response .The cyclic voltammograms of the redox couple 0.8mM potassium ferrocyanide at scan rate 0.1V/s at a bare, MWCNT, f-MWCNT and f-MWCNT-GOT were shown in Fig.(3-124) .



Figure (3-124) Cyclic voltammograms for modified electrodes MWCNT, f-MWCNT, f-MWCNT-GOT and bare in 0.5mM ferri/Ferrocyanide in 0.1M KCl supporting electrolyte.



The peak separations and anodic (I<sub>pa</sub>) and cathodic (I<sub>pc</sub>) peak current height are also illustrated in Fig. (3-125) and (3-126) respectively. The f-MWCNT electrode, yielded good reversibility with  $\Delta E_p$  values of 0.14V and higher peak current I<sub>pa</sub> values of 14.07 µA. This behavior was attributed to the good charge transfer of the electrode. The redox peak current of ferro couple for plain MWCNT was lower than the bare SPCE although the peak separation for MWCNT is lower. This may belong to absence of functionalities that accelerate electron transfer. Thus the peak current was dependent in MWCNT being treated or not. The modified SPCE with f-MWCNT-GOT was exhibited electron-transfer towards ferri/ferro, with a  $\Delta E_p$ value of 0.17V.

The values of  $\Delta E_p$  were lower than the  $\Delta E_p$  for vertically aligned (VA) CNTs and horizontally/randomly oriented (HRO) CNTs by 163 mV and 277 mV [264] respectively. The reduction current of f-MWCNT -GOT nanocomposites was a little higher than f-MWCNT. The anodic peak potentials that caused to oxidation ferricyanide at the modified electrodes MWCNT, f-MWCNT and f-MWCNT-GOT are almost the same 0.21, 0.20 and 0.21 V respectively and lower than bare SPCE (E<sub>pa</sub> 0.24 V).



Figure (3-125) The seperation peak potential for bare , modified electrodes MWCNT,f-MWCNT and f-MWCNT-GOT/SPCE in 0.8mM ferri/ferrocyanide



Figure (3-126) Anodic and cathodic peak current densities for bare MWCNT,f-MWCNT and f-MWCNT-GOT/SPCE in 0.8mM ferri/ferrocyanide

The half-wave potentials  $E_{1/2}$  were determined from the anodic ( $E_{pa}$ ) and cathodic ( $E_{pc}$ ) peak potentials according to equation 1.

$$E_{1/2} = (E_{pa} + E_{pc})/2$$
 (3-41)

And the peak current ratio  $I_{pa}/I_{pc}$  was for all the modified electrodes MWCNT, f-MWCNT, f-MWCNT-GOT and bare were listed in Table (3-17).

Table (3-17) Electrochemical parameters characterizing the redox couple0.8mM ferrocyanide on bare SPCE, MWCNT, MWCNT/GOT, F- MWCNT /GOT and F-CNT/GOT electrodes at scan rate 0.1 Vs<sup>-1</sup>.

electrodes	$E_{1/2}(V)$	$I_{pa}/I_{pc}$
Bare	0.12	0.87
MWCNT	0.125	1.03
f- MWCNT	0.13	1.34
f-MWCNT -GOT	0.125	0.94



## 3.5. The effect of pH

The electrochemical behaviors of ampicillin antibiotic were dependent on the pH value of solution [28, 29]. To evaluate the optimum pH for ampicillin detection on the surface of the modified electrode, its electrochemical behavior was investigated in the buffered solutions with various pHs in the range of 2-8. A buffered solution of pH 2 was chosen as an optimum value in order to obtain the best sensitivity in all voltammetric measurements fig.(6).



**Figure 6.** Electrochemical behavior of AMP at different pH value in PBS at sweep rate  $0.1 \text{ V s}^{-1}$ .

# **3.11.2** The modified SPCE with MWCNT and its composite for the detection test of AMP.

For probing the feature of the modified surface of SPCE, CV was employed to investigate the electrochemical properties of each modified electrode. For bare SPCE very weak andic peak current was observed in pH2 was  $(0.6\mu A)$  and in presence of AMP  $(0.67 \mu A)$  Fig. (3-127).





Figure (3-127) Cyclic voltammograms for bare-SPCE in presence AMP in buffer pH2 and in PBS pH2 only.

Fig. (3-128) showed the CVs of modified electrodes, MWCNT, f-MWCNT and f-MWCNT-GOT in PBS buffer (pH = 2) at scan rate 0.1V/s. MWCNT-SPCE showed oxidation peak of AMP at 0.12 V with oxidation current about (6.273 $\mu$ A). The current of AMP clearly increased with f-MWCNT-SPCE, 46.072  $\mu$ A for anodic and -19.26  $\mu$ A for cathodic, indicating that the carboxylic group significantly enhanced the electron transfer between AMP and surface of f-MWCNT-SPCE with redox peaks at -0.19 and 0.13 V respectively. Based on the comparison in terms of the peak current, SPCE modified with f-MWCNT-GOT nanocomposite produced the highest peak currents (105.279  $\mu$ A at potential 0.19V) for anodic and (-23.976  $\mu$ A at potential -0.36V) for cathodic. Since MWCNT and f-MWCNT have the lowest peak current, it was not considered further in the study.





Figure (3-128) Cyclic voltammograms for bare and modified electrode MECNT, f-MWCNT and f-MWCNT-GOT in presence and absence of 0.1mM AMP.

## 3.11.3 Effect of scan rate

Cyclic voltammograms of the AMP were recorded at different scan rates (from 0.03 to 0.18 V s<sup>-1</sup>) Fig. (3-129).



Figure (3-129) Cyclic voltammograms for modified electrode f-MWCNT-GOT in presence of 0.1mM AMP at different scan rates.



The dependence of anodic peak current  $(I_{pa})$  and cathodic peak current  $(I_{pc})$  on the scan rate showed, a linear relationship .Fig (3-130) illustrates that the anodic and cathodic peak currents  $(I_p)$  were linearly dependent on scan rate (v).



Figure (3-130) Variation of the peak current  $(I_{pa})$  and  $(I_{pc})$  with the sweep rate for 0.1mM AMP at f-MWCNT-GOT nanocomposite modified SPCE.

The peak heights for the anodic signal was higher than the cathodic signal  $I_{pa}/I_{pc} \neq 1$ . Also, the potential difference between the anodic and cathodic potential increased with varying scan rates between 0.03 and 0.18V.s<sup>-1</sup>.As the reverse wave is noticed at  $\Delta E_p > 0.4V$  [265]for this AMP have irreversible charge transfer process  $E_{irr}$  as shown in table (3-18).

Scan rate (V.s <sup>-1</sup> )	$E_{pa}-E_{pc}(V)$	I <sub>pa</sub> /I <sub>pc</sub>
0.03	0.25	4.4
0.05	0.34	4.5
0.07	0.39	4.52
0.09	0.48	3.35
0.12	0.49	3.24
0.14	0.54	4.38
0.16	0.63	4.18
0.18	0.55	4.68

Table (3-18) Potential separation  $\Delta E_p$  with ratio of anodic and cathodic peaks current for f-MWCNT-GOT in presence of AMP at different scan rates.



According to the equations (3-42) for an irreversible electron transfer ET [265]  $\partial Ep/\partial \log v = 1.15RT/F\alpha$  (3-42)

And from fig (3-131) The values of the electron transfer coefficient  $\alpha$ , which have been estimated from the peak potential slope, and found to be 0.15 this diminished due to breaking bonds (high activation energy).



Fig (3-131) variation of potential with log scan rate

As for the irreversible electrode process of the oxidation reaction of AMP, the Laviron's equation (equ.4) [36] was used to estimate n and  $k_s$  values as follows:

$$E_{p}=E^{0}+(2.303RT/\alpha nF) \log(RTk_{s}/\alpha nF)+(2.303RT/\alpha nF) \log v ] \qquad \dots (3-43)$$

Where  $\alpha$  is the electron transfer coefficient,  $k_s$  is the standard rate constant of the surface reaction, v is the scan rate, n is the electron transfer numbers and  $E^0$  is the formal potential.  $K_s$  and n values can be concluded from the intercept and slope of the linear plot of  $E_p$  with respect to log v fig. (3-131), if the value of  $E^0$  was known. The  $E^0$  value at f-MWCNT-GOT nanocomposite of the modified SPCE can be deduced from the intercept of Ep *vs.* v fig.(3-132) plot on the ordinate by extrapolating the line to v=0. Knowing  $E^0$ , and from the graphical representations of  $E_p$  *vs.* Log v for AMP fig. (11 A)



Figure (3-132)The variations of peak potential vs. v at f-MWCNT-GOT/SPCE.


The value of n=2 obtained from the slope indicated that two electron was involved in the oxidation of AMP at the modified SPCE with f-MWCNT-GOT nanocomposite. Also the values of  $k_s$ =432.68 cm.s<sup>-1</sup> was obtained from the intercept. This high enhancement in value of  $k_s$  compared with other modified electrode clearly demonstrate that the introduction of a nanocomposite f-MWCNT-GOT in the surface of electrode opens an efficient ET between AMP and the modified WE.

Linear relationships were observed between log Ip and log v over the scan range 0.03-0.18 V s<sup>-1</sup> with slope of 0.6442 and 0.6781 respectively as shown in Fig. (3-132). The slope of the straight lines are intermediate value between 1 the theoretical value of the adsorption controlled electrode process and the theoretical value of 0.5 which was expressed for the diffusion controlled electrode process, this indicated that the electrode process was controlled simultaneously both by diffusion and adsorption [226]for redox process.



Figure (3-131) Plot of log current vs. log scan rate v (V/s). **3.11.4 DPV measurements** 

The DPV of the f-MWCNT-GOT modified SPCE in the presence of various concentration of AMP are shown in Fig. (3-132) the oxidation of AMP was accompanied by an increase in the anodic current which could significantly improve the detection limits of electrochemical measurement.





Figure (3-132) Differential pulse voltammogram for modified SPCE with f-MWCNT-GOT nanocomposite in presence of different concentrations of AMP.

Under optimum experimental conditions the calibration curve was obtained. The peaks current increase linearly with the increase of AMP concentration in range of  $1.52 \times 10^{-7}$  to  $1.46 \times 10^{-5}$  M Fig. (3-133).



Figure (3-133) The relationship between current and the concentration of AMP.



## Chapter three

The detection limit was estimated as  $1.46 \times 10^{-7}$ M using the same equation (3-20) according to this equation the f-MWCNT-GOT modified electrode has lower detection limit.

## **3.11.5** Surface characterization of the surface of modified SPCE with f-MWCNT and its composite f-MWCNT-GOT.

Fig. (3-134) shows the SEM image of f-MWCNT. The SEM image reveals that MWCNT has well distribution on the SPCE surface. From Fig. (3-135) it was clearly that the composite is well distributed even though some MWCNT still visible. There are several proposals that clarify the mechanism of dispersed the MWCNT with nanosheets. S.Chen et al [266] proposed the sheets can wrap around the CNTs while M.Shin et *al* [267] proposed the CNTs are attached to the edges and surfaces of the graphene sheets.



Figure (3-134) SEM image of surface of f-MWCNT







The importance of the carbone nanomaterials are daily increased in many research areas and its uses as a sensor. Also, because they are cheap, efficient, fast and give the results without further laboratory preparation especially.

Therefore in this work we successfully prepared many carbone nanomaterials (GO and RGO) and functionalized with 5-substituted 4-amino, 3-mercapto 1, 2, 4 Triazole (ASTT) groups (GOT and RGOT), moreover the above carbone materials made a composite with MnO<sub>2</sub> nanorod (GOTM and RGOTM). On other hand a commercial multiwalled CNT, was carboxylate with strong acid (f-MWCNTs) and nanocomposites with GOT was prepared. All the above carbone nanomaterials were characterized with FT-IR and XRD and compatible with references. The morphology of the GO, RGO nanosheets and MnO<sub>2</sub> nanorods were studied with AFM technique. The GO shows sheet thickness 3.71 nm and this represented mono sheet thickness, which means a very good syntheses of the carbone nanomaterial and useful for later preparation of the nano compounds, the RGO and all the functionalized material depended on this material, on other hand the image of the MnO<sub>2</sub> nanorods showed the development of the rods from the spherical MnO<sub>2</sub> nanoparticles and this image is very uncommon in the references, also the number of moles of KMnO<sub>4</sub> played important role on the preparation and controlled diameter of nanorods.



The prepared carbone nanomaterials showed different behaviors and values in the electrical measurements, the real permittivity was in the following sequence from the high to the low value at 400 Hz GOT 27187345 >RGO 8598418.079> RGOT 8290508.47>GOTM 5970998 > GO 8376.648 > RGOTM 0.459548023 > f-MWCNT -17346892.7.

On other hand, the electrical conductivity at 400Hz was found in the following sequence from the high to low values.

f-MWCNT-GOT 18.86 > GOT 0.67 > RGO 0.21 > RGOT 0.17 > GOTM 0.15 > GO 0.0002 > RGOTM 1.22E-09

The electrical conductivity of GOT was higher than the RGO and RGOT, this result may be attributed to the strong reduction process during the functionalization of GO with TCH, and that could supported with results of XRD of GOT, in fig.3-17 the diffraction at  $20 \ 11^{\circ}$  was disappeared this indicated a reduction process and at the same time appeared the 002 diffraction again in  $20 \ 27.3^{\circ}$  this may be due to the rearrangement of the sheets in form nearly like in graphite structure and caused the high conductivity values. All the prepared carbone nanomaterials examined with cyclic voltammetry technique to investigate the sensing ability on different antibiotics materials the prepared carbone nanomaterials showed a relatively different cathodic and anodic current values, which are due to the different electrochemical activities and the speed of electron movement between the examined antibiotic and the working electrode. The following carbone nanomaterials show a sensing and the detection of the antibiotics.

f-MWCNT-GOT composite detected AMP

f-MWCNT detected AMP

GOT detected AMOX

RGOT detected AMP

GOTM detected TET

RGOTM detected AMP

The above carbone nanomaterials were from top to bottom match with the electrical conductivity values from the highest to the lower one. The f-MWCNT-GOT composite and f-MWCNT showed the highest current peak in comparison with other materials.



## 3.13 Future Study

1- The obtained results encourage the researcher to work in the wider area of the different families of antibiotics.

2- Development of new carbone nanomaterials with other functional groups were not used in this work.

3- Functionalization of the carboxylate MWCNT with TCH and examine the LOD values of different antibiotic and agricultural pesticides.

4- Study the effect of the real and imaginary values of permittivity and the electrical conductivity of the prepared carbone nanomaterials on the LOD values.

5- The prepared carbone nanomaterial in this work can be studied the environmental pollution with agricultural pesticides, those containing amide groups.

6- Preparation of new carbone nanomaterials with nano-metal oxide, nano metal and or conductive polymer, to detect the different organic and inorganic materials.

7- Functionalize the prepared carbone nanomaterials with azo compounds and studying the photo electric effect of the cis-trans isomerization in photo electric cells.

8. Study the aqueous suspension of the reduced graphene oxide and graphene oxide functionalized with thiocarbohydrazide RGOT, GOT respectively alone or with MWCNT by incorporate enzymes to using them in biological applications.

9. Use this novel functionalities with hydrogel to study drug delivery.

10. Use this nanomaterials as adsorbent surface to many pollutants.

11. Study Influence of this novel functionalities on Dielectric Behavior of some polymers



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